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SUPRAGLOTTIC LARYNGECTOMY: FUNCTIONAL AND ONCOLOGIC RESULTS

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TRACHEOTOMY IN CHILDREN WITH JUVENILE-ONSET RECURRENT RESPIRATORY APILLOMATOSIS: THE CHILDREN'S HOSPITAL OF PITTSBURGH EXPERIENCE

BASALOID SQUAMOUS CELL CARCINOMA OF HE HEAD AND NECK: CLINICOPATHOLOGICAL FEATURES AND DIFFERENTIAL DIAGNOSIS

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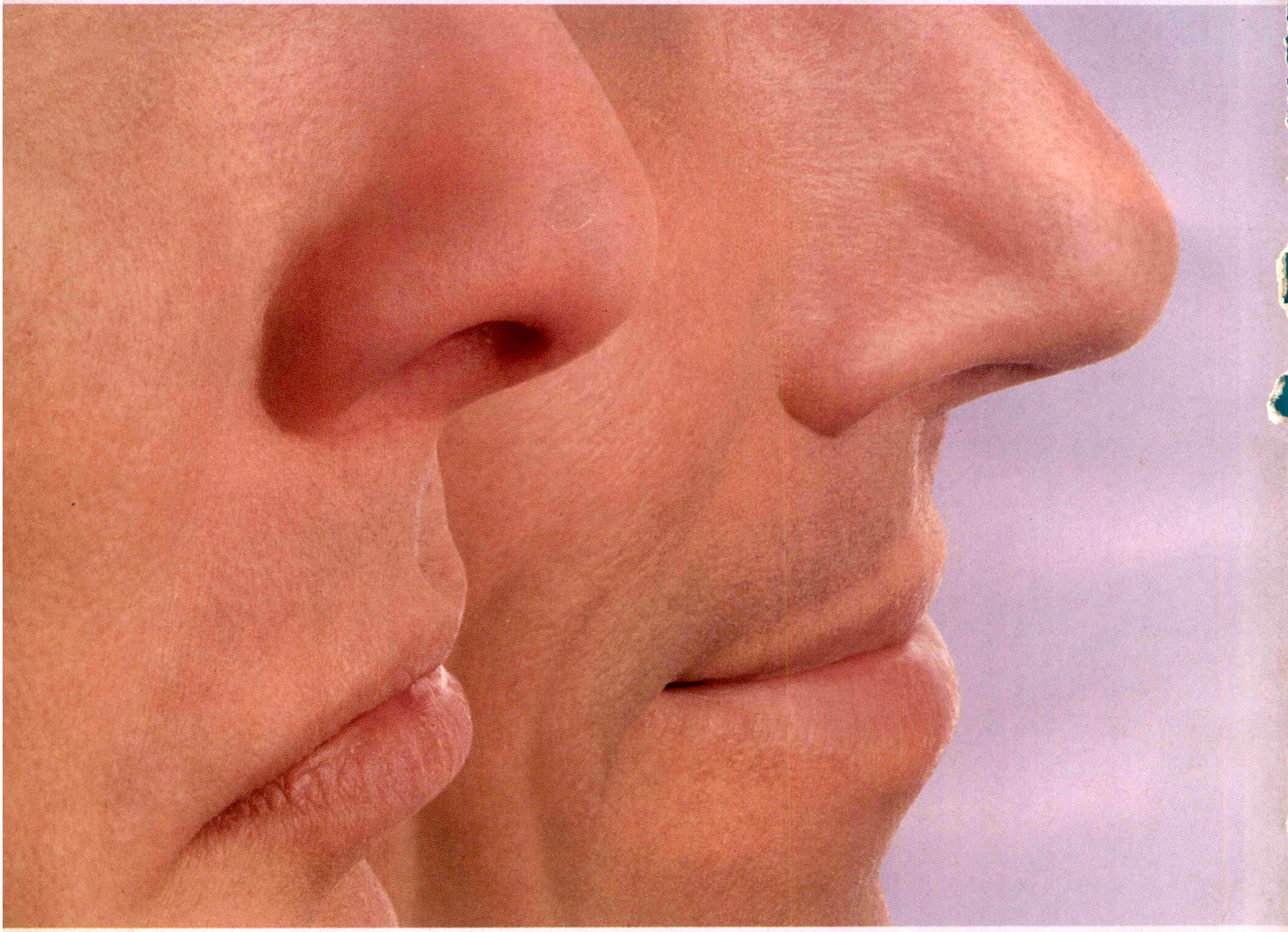

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TRACHEOTOMY IN CHILDREN WITH JUVENILE-ONSET RECURRENT RESPIRATORY PAPILLOMATOSIS: THE CHILDREN'S HOSPITAL OF PITTSBURGH EXPERIENCE

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Despite the risk of airway obstruction, tracheotomy has been viewed with trepidation in the management of recurrent respiratory papillomatosis (RRP). The literature suggests that the injury associated with the tracheotomy site may initiate the progression of disease to the distal airway. Alternatively, patients who require tracheotomy for RRP may be predisposed to distal spread because of more aggressive disease. In an effort to clarify this issue, we reviewed the Children's Hospital of Pittsburgh experience with 35 patients with RRP between 1984 and 1994; 13 patients received tracheotomies. Tracheotomy patients presented at a younger age with more widespread disease, often involving the distal airway prior to tracheotomy. Although distal spread occurred in 50% of patients, it was generally limited to the tracheotomy site. Overall, outcome in the tracheotomy group was satisfactory. Complications related to the tracheotomy were rare. We conclude that tracheotomy is an appropriate option for significant airway compromise in patients with RRP.

KEY WORDS — recurrent respiratory papillomatosis, tracheotomy.

INTRODUCTION

Papillomata of the respiratory tract are benign tumors that may arise in both children and adults. Respiratory papillomata are most frequently located at the glottis, typically manifesting symptoms of hoarseness or airway obstruction. However, lesions may be found at any site in the upper aerodigestive tract. Papillomata may be solitary, multiple, or recurrent. Recurrent respiratory papillomatosis (RRP) encompasses a spectrum of disease states ranging from infrequent recurrences of solitary lesions to a persistent, disseminated, and at times fatal form. Although papillomata are the most common benign neoplasms of the larynx in children, RRP is a relatively uncommon disease. In the last decade, significant advances in the pathogenesis of this disease have been elicited. The causative agent in RRP is the human papillomavirus (HPV), specifically types 6 and 11. After decades of speculation, HPV has now been identified in the vast majority of respiratory papillomata by a number of methods, including Southern blot analysis, in situ hybridization, and the polymerase chain reaction.[1-3] In children, the source of inoculation of HPV appears to be condyloma of the anogenital tract of the mother, which have been demonstrated to have a similar viral causation. Approximately 60% of children with respiratory papillomatosis are born to mothers with genital papillomata during pregnancy or parturition.[4] The precise mode of transmission remains uncertain, although direct contact during parturition or infection in utero is likely, rather than transmission during the postpartum period. Currently, the standard therapy for RRP is sequential laryngoscopic and/or bronchoscopic excisions by means of the carbon dioxide (CO_2) laser.[5]

Unfortunately, many facets of RRP are not as well characterized. One of the more perplexing issues that remains unresolved is how to predict the course and severity of disease in an individual patient. Previously, "juvenile-onset" papillomatosis was believed to represent a relatively aggressive disease that typically remitted at puberty, while "adult-onset" papillomatosis implied a relatively benign course. Despite attempts to characterize patients on the basis of the age at onset or the early pattern of disease, spontaneous remissions and exacerbations are common and often fall outside the range of expected behavior.[6] Furthermore, the influence of specific viral subtypes, other viral or environmental cofactors, and the interaction of the immune system on the ultimate progress of the disease are still being explored.[7,8] Finally,

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the indications, benefits, and risks of various adjunctive medical therapies are largely undetermined, although many trials have been completed or are in progress.[9-11]

In a minority of patients, the rapid and extensive growth of papillomata may result in episodes of life-threatening airway obstruction. In these cases, tracheotomy may be required to provide a reliable airway during extirpation of papilloma and maintain a margin of safety against future obstructive events. However, a number of series have demonstrated that tracheotomy in patients with RRP may be associated with the development of disease at the tracheotomy site.[12-16] Papillomata introduced into the trachea may then progress to involve the bronchi, and in extreme cases the lung parenchyma. Thus, the physician managing the patient with severe obstructive RRP faces a difficult dilemma: the desire to protect the airway, weighed against the possibility of initiating a cascade that could ultimately accelerate the disease process.

The purpose of this study was to provide additional insight into the impact of tracheotomy on the course of RRP in pediatric patients. The patient characteristics, frequency of procedures, and distribution of disease in tracheotomized (T) and nontracheotomized (NT) patients were compared. Additionally, the outcome of therapy was compared in patients whose courses were complete.

METHODS

A review of the medical records of all patients admitted to the Children's Hospital of Pittsburgh with the diagnosis of RRP in the 10-year period between January 1984 and January 1994 was performed. The charts of 43 patients with histopathologically confirmed respiratory papillomata were available for evaluation. Patients with insufficient data (eg, only one procedure, no follow-up) to yield conclusions were excluded from the study, rendering 35 patients suitable for review. Thirteen (37%) of these patients received tracheotomies. Ten of the tracheotomies were performed at Children's Hospital of Pittsburgh, while 3 were placed at outside institutions. The age at onset, symptoms, and past medical history were recorded. For various reasons, the birth history was not uniformly available, nor was documentation regarding maternal condylomata. The initial location of disease was determined by examination, or by records received from the institutions in which the patients were originally treated. All of these patients had repeated endoscopic airway procedures, typically employing jet ventilation and inhalational anesthesia. Carbon dioxide laser and cold instrumentation were utilized as appropriate for removal of papillomata. The procedures were performed in accordance with the philosophy of the senior author (S.E.S.); since surgery was not curative, the goal of each procedure was to establish a satisfactory airway while maximally preserving laryngeal anatomy and function. Complete papilloma removal was accomplished when possible, but residual disease was permitted to remain when necessary to protect against disabling scarring in anatomically vulnerable areas, such as the anterior commissure and posterior glottis. Each operative report contained information regarding the presence or absence of papilloma at individual subsites of the airway, consisting of the supraglottis (including the false vocal cords), glottis, subglottis, trachea, and bronchi. The subglottis was defined as extending from the inferior aspect of the true vocal cords to the inferior edge of the cricoid cartilage. Stomal disease was defined as disease at the tracheotomy, or within 1 cm of the distal end of the tracheotomy tube. Tracheal referred to all other disease, not associated with the tracheotomy, up to and including the carina. Papillomata at less commonly diseased sites, such as the palate or tongue, were noted when present. Remission of disease was defined as a pattern of decline in disease intensity followed by at least two consecutive examinations documenting the absence of papillomata during a 6-month period. Follow-up after remission ranged from 2 months to 8 years. Because relapses of RRP have been noted many years after disappearance of papillomata, we have refrained from designating any patient as "cured."

RESULTS

Age at Presentation. The majority of patients received a diagnosis of respiratory papillomatosis at endoscopy within 3 months of the onset of symptoms. Patients who ultimately required tracheotomy presented for evaluation at an average age of 21.4 months, compared to NT patients, who did not present until an average age of 52.3 months. Thus, patients who went on to require tracheotomy presented at an earlier age, which may be an indication of more aggressive disease early in the course, or simply an earlier onset of disease.

Age at Tracheotomy. The average age at which tracheotomy was performed was 2 years 7 months.

Presenting Symptoms. Hoarseness and airway compromise were the most common presenting symptoms in this series. In the group of patients requiring tracheotomy, 6 of 13 (46%) presented with stridor or recurrent croup, while 7 of 13 (54%) were noted to have hoarseness. Of note, 2 of these patients had "total" aphonia, which had been present since birth.

In contrast, 18 of 22 (82%) patients in the NT group presented with hoarseness. Three (14%) patients in this group presented with stridor, although in 1 patient the cause of the stridor appeared to be subglottic stenosis, with papillomata noted as an incidental finding. One patient presented with a nasal obstruction secondary to nasopharyngeal papillomatosis, and on subsequent endoscopy was found to have papillomata involving the larynx as well.

Sex and Race. Of the T group, 9 (69%) patients were white, 2 (15%) were African-American, 1 was Hispanic, and 1 was Asian. There were 7 (54%) girls and 6 (46%) boys in this cohort.

In patients without tracheotomy, 19 (86%) were white, while 3 (14%) were African-American. There were 13 (59%) girls and 9 (41%) boys in this group.

Adjunctive Therapy. Only 1 patient in each group received medical therapy in conjunction with surgical treatment of RRP. Both of these patients received interferon. The T patient, who had presented with widespread papillomata, also underwent therapy with bleomycin and methotrexate. This patient eventually died secondary to extensive disease. No conclusions can be drawn regarding the use of adjunctive therapy in these patients.

Number of Procedures. Over a 10-year period, 994 laryngoscopic or bronchoscopic procedures were performed for the management of RRP in the 35 patients included in this study. Of those, 668 were performed in patients in the T group. As evidence of the broad range of disease severity encountered in juvenile patients with RRP, the number of procedures performed on an individual patient ranged from 2 to more than 100. The average number of procedures per month was calculated for T patients both before and after tracheotomy. Prior to the tracheotomy, the average number of procedures per month was 0.95; following the tracheotomy, patients underwent an average of 0.85 procedures per month. There did not appear to be any notable difference in the thoroughness of the surgical procedures before or after tracheotomy. Thus, the presence of the tracheotomy did not appear to accelerate the growth of papillomata. Patients who did not require tracheotomy averaged 0.62 procedures per month. The requirement for more frequent procedures in the T patients may be an indication of more aggressive disease in this group.

Initial Site of Disease. The majority of the RRP patients in this series had disease involving the glottis. In the NT group, 14 patients had disease located at only one site. Eighteen of the patients had disease that involved the glottis; of these, 10 had disease isolated to the glottis, while 7 also had supraglottic disease (usually the false vocal cords), and 1 had papillomata involving the subglottis. Three patients had isolated supraglottic disease, and 1 had isolated subglottic disease. No patient in the NT group had disease distal to the subglottis at the time of presentation.

In contrast to the NT group, the patients with tracheotomy were much more likely to have disease at multiple sites at the time of presentation. Only 1 of 13 patients had disease isolated to one laryngeal site. Six patients (46%) had disease involving the supraglottis, glottis, and subglottis or trachea at the time of presentation. Disease distal to the glottis was also much more common among the 13 patients who went on to require tracheotomy. Five patients (38%) presented with disease involving the subglottis, 3 patients had tracheal disease, and 1 patient (No. 9) had disease involving the main stem bronchus and lung.

Disease Progression. In the NT group, 2 patients (9%) had disease that progressed to involve the trachea; 1 had subglottic disease at presentation, while the other developed subglottic disease in association with the tracheal papillomata. Seven of the patients in the T group (54%) demonstrated progression of disease beyond the initial site of involvement. In 6 of these patients, none of whom had subglottic disease at the time of presentation, progression was isolated to the tracheotomy site, without involvement of the distal airway. In the 1 patient with subglottic disease at presentation, isolated papillomata were subsequently noted at the carina and main stem bronchus orifice. Disease progression was noted between 6 months and 1 year following tracheotomy.

Age at Decannulation. Ten of the 13 patients who required tracheotomy achieved sufficient resolution of their disease to be decannulated. The average age at decannulation was 6 years 4 months. In 5 patients, the disease was noted to have resolved prior to decannulation.

Duration of Disease and Age at Remission. It is difficult to interpret data regarding duration of disease in this review, since many of the patients have continued to receive therapy for persistent disease for intervals ranging from 4 months to 12 years. Of the 13 patients who required tracheotomies, 9 patients achieved remission at an average age of 6 years 4 months. As noted above, 5 of the patients demonstrated resolution of their disease while the tracheotomy tube was still in place. The remaining 4 had resolution within 6 months of decannulation. Papillomata associated with the tracheotomy site invariably resolved within 1 to 2 months of resolution of the primary disease. In the NT group of 22 patients, 7 patients achieved remission at an average age of 5 years 8 months. The remaining patients continue to undergo treatment for RRP.

Complications. One patient in the T group (No. 9) died of disseminated disease. This patient had fulminant distal airway and pulmonary disease prior to tracheotomy. Four patients in the NT group developed anterior glottic webs that responded to simple measures such as laser excision or dilation. One patient in this group also developed a posterior glottic web that was successfully treated with laser excision. A single patient in the T group developed an anterior glottic web that required treatment with median thyrotomy and keel placement; this patient has been successfully decannulated.

Six of the patients in the T group required urgent airway intervention for severe obstruction prior to placement of the tracheotomy; several patients had multiple episodes. Only 1 patient in the NT group required urgent airway intervention because of obstructive disease. Complications specifically related to the tracheotomy appeared to be few; 1 patient developed bilateral pneumothoraces following tracheotomy that resolved after conservative measures. Several children were noted to have granulation tissue at the stoma site.

DISCUSSION

The propensity for tracheal involvement in RRP patients with tracheotomy has been well established in the literature. In 1964, Majoros et al[12] reported subglottic disease in 56% of patients with tracheotomy, compared to 8% in their nontracheotomized patients. Cole et al,[13] in a review of the literature as well as their own experience, documented incidences of tracheal spread ranging between 7% and 76%. Cohen et al[14] related their low incidence of tracheobronchial spread largely to their ability to limit the use of tracheotomy.

The likely reservoir for persistent HPV infection in patients with RRP is clinically uninvolved areas of the lower airway.[3] The presence of HPV has also been demonstrated in airway mucosa following remission of disease.[17] Kashima et al[16] have noted the predilection for papilloma to grow at the junctions between ciliated respiratory mucosa and squamous epithelium; such areas may be found on specific surfaces of the true and false vocal folds. Ciliated-squamous junctions may also be created by injury to the airway mucosa, such as the tracheotomy site.[16] Consequently, the tracheotomy may promote an "activation" of the latent HPV reservoir in the trachea. Kashima et al also note the propensity of disease introduced into the trachea at the site of tracheotomy to progress in a stepwise fashion to involve the more distal airway. Thus, both epidemiologic and "laboratory" data support the avoidance of tracheotomy in patients with RRP whenever possible. As an option, Tucker[18] recommended "double-barreled" (diversionary) tracheotomy to prevent distal spread in patients who require tracheotomy.

An alternative, and perhaps less widely accepted, concept regarding distal spread is that patients who require tracheotomy have more aggressive disease from the outset; such patients are thus predisposed to develop tracheobronchial disease.[16] A comparison of the T and NT patients in this series tends to support this proposition. Patients who eventually required tracheotomy presented at a much earlier age, were more likely to manifest severe obstruction, and required more surgical procedures than the NT group. The T patients were also more likely to have widespread disease involving the distal airway.

A second issue addressed in this review is the impact of tracheotomy on the course of the disease and the ultimate outcome. Tracheotomy was associated with distal progression of disease in 53% of patients, consistent with other series.[13] However, only 1 patient, who had subglottic disease prior to tracheotomy, had progression beyond the tracheotomy site. In the remaining 6 patients, disease was confined to the site of injury associated with the tracheotomy tube. Furthermore, papilloma involving the trachea tended to resolve at the same time as laryngeal disease, often with the tracheotomy tube still in place. Ten of the 13 patients with tracheotomies achieved remission, at an average age of under 7 years. While it is not possible to conclude that the tracheotomy did not result in prolongation of clinical disease in these patients, the age at which the T patients in this series achieved remission compares favorably to those in other series of patients with juvenile-onset RRP.

CONCLUSIONS

Patients with RRP who require tracheotomy manifest earlier onset of disease, which appears to be more aggressive and widespread than in their NT counterparts. This study reaffirms the observation that tracheotomy is associated with distal involvement of laryngeal papillomatosis. However, distal disease often predated the tracheotomy. Furthermore, when progression occurred, it tended to be confined to the area of injury surrounding the tracheotomy, and was not associated with dissemination to the distal trachea or bronchi. Finally, remission was achieved in the majority of T patients at an age that agrees favorably with findings in other series of NT patients with RRP. Tracheotomy-related complications were few.

Only a randomized prospective study could demonstrate with assurance the true impact of tracheotomy on children with RRP; ethical and logistical issues make such a study nearly impossible. We feel certain that careful management without a tracheotomy is the best option for children with RRP, and will continue to manage RRP patients in this fashion whenever possible. It must be emphasized that the patients in this series who received tracheotomies while under our care were felt to have severe disease that could not be managed safely without a tracheotomy. The results of this review suggest that the adverse effect of tracheotomy on the outcome of these patients may be overestimated. In fact, tracheotomy was lifesaving in a number of our patients. Additional benefits were not easily measured; for example, a number of parents have offered that the tracheotomy provided them with relief from the fear of rapidly progressive airway obstruction. Induction and maintenance of anesthesia for removal of papillomata became a less menacing process. We contend that no child should be forced to endure a tenuous, life-threatening airway because of papillomata.

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IMMUNOCYTOCHEMICAL STUDY OF PROTEOGLYCANS IN VOCAL FOLDS

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We evaluated the proteoglycan composition of normal vocal folds using immunocytochemical techniques. Frozen sections of 14 normal cadaveric vocal folds were obtained within 12 hours of death and sectioned immediately. Vocal fold sections were stained with antibodies against keratan sulfate, chondroitin sulfate, heparan sulfate proteoglycan (HSPG), decorin, and hyaluronate receptor. We found that the lamina propria has diffuse staining of fibrillar components with keratan sulfate and decorin. Intense staining was observed in the vocal ligament area with keratan sulfate. The HSPG was localized to the basement membrane zone. Chondroitin sulfate, HSPG, and hyaluronate receptor were detected in the cytoplasm of interstitial cells with immunocytochemical characteristics of macrophages. The keratan sulfate distribution suggests that fibromodulin may be significant in normal vocal folds. Production of HSPG and probably versican occurs in macrophages and fibroblasts in the lamina propria.

KEY WORDS — collagen, extracellular matrix, glycosaminoglycans, proteoglycans, vocal folds.

INTRODUCTION

Benign laryngeal lesions such as polyps, nodules, scars, and polypoid corditis may represent a disturbance in the balance of the extracellular matrix constituents of the vocal folds. To evaluate this hypothesis, it is first necessary to characterize the components contributing to the normal extracellular matrix in the lamina propria.

The vocal fold lamina propria consists of two important tissue components: a cellular component and extracellular matrix. As the cellular component is sparse, it appears that the extracellular matrix has the determining biochemical role.[1] The extracellular matrix is composed of two families of macromolecules: collagen and elastins, which provide the fibrous scaffolding of the lamina propria; and the substance of the interstitium or "filling" between the fibrous scaffolding — the proteoglycans and structural glycoproteins.[2]

Proteoglycans are ubiquitous substances that have been implicated in a wide variety of processes, such as cell adhesion and migration, matrix assembly, growth factor sequestration and regulation, immune function, binding of plasma proteins, and control of thrombogenesis.[3-5]

Proteoglycans are composed of glycosaminoglycan chains covalently attached via a linkage protein to a protein core.[4] Glycosaminoglycans are linear polymers and include keratan sulfate, chondroitin sulfate, dermatan sulfate, heparan sulfate, and hyaluronic acid. Hyaluronic acid differs from other glycosaminoglycans in that it is not covalently attached to proteins.[6]

Three subgroups within the extracellular matrix proteoglycan family have been identified: 1) small, interstitial matrix proteoglycans — decorin, biglycan, and fibromodulin; 2) large, aggregating chondroitin sulfate proteoglycans — aggrecan and versican; and 3) heparan sulfate proteoglycans. This classification is based on the similarities of the protein cores and glycosaminoglycan chains.[6,7] Each of the proteoglycans has characteristic features in relation to glycosaminoglycan chains, structure, function, and localization in other tissues, as summarized in the Table.[3,6-23]

Proteoglycans have not received as much attention as the other components of the lamina propria of vocal folds. Understanding the normal distribution and interactions of these macromolecules may be a guide to discovering the function and interaction of components of the extracellular matrix and eventual correlation with various disease states of the vocal folds. We therefore undertook this descriptive study of normal vocal fold proteoglycans.

MATERIALS AND METHODS

Tissues Studied. Frozen sections of 14 normal

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Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, May 2-4, 1995.

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CHARACTERISTICS OF PROTEOGLYCANS

Proteoglycan	*Glycosaminoglycan*	*Size (kd)*	*Function*	*Localization*
Aggrecan	CS and KS	2,500	Binds hyaluronic acid[16] Provides strongly hydrated, space-filling gel[16] Modulates proliferative and metabolic activities of chondrocytes and fibroblasts[9] Plays structural role in development of cartilaginous structures[11]	Not found in LP of VF Found in ECM of cartilage, tendon, sclera, aorta, and bone[17,18]
Versican	CS	>1,000	Binds hyaluronic acid[12] Has ability to fill space, bind and organize water molecules[6] Plays role in hydration of cartilage[6]	Found in macrophages and fibroblasts in LP of VF Expressed by fibroblast cells Isolated from various cell cultures and tissue sources, such as intima of aorta[19]
Biglycan	CS and DS (2 chains)	50-200	Binds transforming growth factor–β in vitro — feedback mechanism regulating synthesis of biglycan[7] Adsorbs to hydroxylapatite crystals after being shed from osteoblasts	Has not been searched for in VF CS: found in fetal or young bone DS: found in articular cartilage, skin, tendon, and sclera[11,20] Found in specialized cell types in developing human tissues, including bone, cartilage, blood vessel endothelial cells, skeletal myofibrils, renal tubular epithelia, and differentiating keratinocytes[3,21,22]
Decorin	CS and DS (1 chain)	50-200	Binds to collagen types I and II in in vitro assays, resulting in delayed fibril formation and formation of thinner fibrils[9,11] Binds to fibronectin and transforming growth factor–β[9]	Found in ECM (fibrillar component) of superficial LP of VF CS: found in developing bone DS: found in cartilage, skin, tendon, and sclera[23]
Fibromodulin	KS	59	Binds to collagen types I and II in in vitro assays, resulting in delayed fibril formation and formation of thinner fibrils[9-11]	Found in ECM (fibrillar component) of intermediate and deep layers of LP of VF Found in vocal ligament area Found in cartilage, tendon, skin, sclera, and cornea[10,17]
HS proteoglycan	HS	55,000 to >400,000	Binds to fibronectin, collagen IV, and laminin[14] Is integral component of basement membranes May play role in tissue morphogenesis, growth control, and differentiation[8]	Found in BMZ of VF Found in macrophages and fibroblasts of LP of VF Found in ECM of cultured epithelial and mesenchymal cells (ie, human lung fibroblasts)[8]
	Hyaluronic acid	100-5,000	Contributes to structure of ECM via interaction with aggrecan and versican Creates highly viscous environment that plays role in molecular exclusion, flow resistance, and tissue osmosis Influences cell adhesion and migration[15]	Found in macrophages and fibroblasts of LP of VF Found in ECM of synovial fluid, umbilical cord, dermis, subcutaneous tissue, and cartilage[13]

CS — chondroitin sulfate, KS — keratan sulfate, DS — dermatan sulfate, HS — heparan sulfate, LP — lamina propria, VF — vocal fold, ECM — extracellular matrix, BMZ — basement membrane zone.

cadaveric vocal folds (no known laryngeal injury, no history of laryngeal intubation) were obtained within 12 hours of death and sectioned immediately. Sections were cut with an Instrumedics attachment to a Tissue Tek Cryostat.

Antibodies. Commercially available antibodies and their dilutions used in this study were as follows.

1. Anti–keratan sulfate (Clone 5D4) (ICN Biochemicals) 1:500.
2. Anti–proteoglycan ΔDI-6S (Clone 3B3) (ICN Biochemicals) 1:50 (epitopes recognized: 0–, 4–, and 6–chondroitin sulfate).
3. Anti–heparan sulfate proteoglycan (Chemicon) 1:20.
4. Anti–hyaluronate receptor (Life Technologies, Inc) 1:100.
5. Anti-decorin (PG40) (Life Technologies, Inc) 1:50.
6. Anti–CD 68 (DAKO) 1:100 (stains cells of macrophage lineage).

Immunocytochemical Method. For the immunocytochemical studies, frozen sections of normal vocal fold were stained with each antibody and compared with the staining of normal skin frozen sections. Antibodies directed against specific proteoglycans and glycosaminoglycans were detected in tissues by means of the avidin-biotin immunoperoxidase method and diaminobenzidine as a substrate.[24] In the case of decorin, an indirect immunofluorescence method was used with a fluorescein-conjugated anti-mouse IgG as the secondary reagent.[25] Controls of skin were used with each staining process.

Interpretation of Results. The intensity and distribution of staining of the peroxidase and immunofluorescence-localized antibodies were evaluated by one observer, who examined vocal fold slides and compared them to control skin slides stained similarly. The intensity of staining in vocal folds was graded as 0+ to 3+ on the basis of its relationship to the control. If the staining of the specimen was as intense as that found in the control, the grade was considered 3+. Weak staining was considered as 0 to 1+. Intermediate grades of staining were considered to be 2+.

RESULTS

Keratan Sulfate and Decorin. We have found that normal vocal fold lamina propria has staining of fibrillar components with keratan sulfate and decorin (intensity of 2+). Decorin was more prominent in the superficial rather than deeper layers (see Figure, A). It is also of interest that a very intense staining of the vocal ligament area occurred with keratan sulfate (intensity 3+; see Figure, B).

Chondroitin Sulfate and Hyaluronate Receptor. Chondroitin sulfate and hyaluronate receptor staining were detected only in the cytoplasm of cells in the lamina propria (intensity 2+ for both; see Figure, C). Many cells that displayed cytoplasmic staining had immunocytochemical characteristics of macrophages. Serial sections stained with antibody directed against CD68 illustrated that many cells (up to 25%) were positive for CD68 and proteoglycans. Remaining cells not staining with CD68 were spindle-shaped and were presumed to be fibroblasts.

Heparan Sulfate Proteoglycan. Heparan sulfate proteoglycan, an integral component of basement membrane in probably all mammalian tissues,[8] was localized to the basement membrane zone of the vocal folds and blood vessels (intensity 2+). In addition, a cytoplasmic localization in macrophages and spindle cells (presumed to be fibroblasts) in the lamina propria was also observed (intensity 2+).

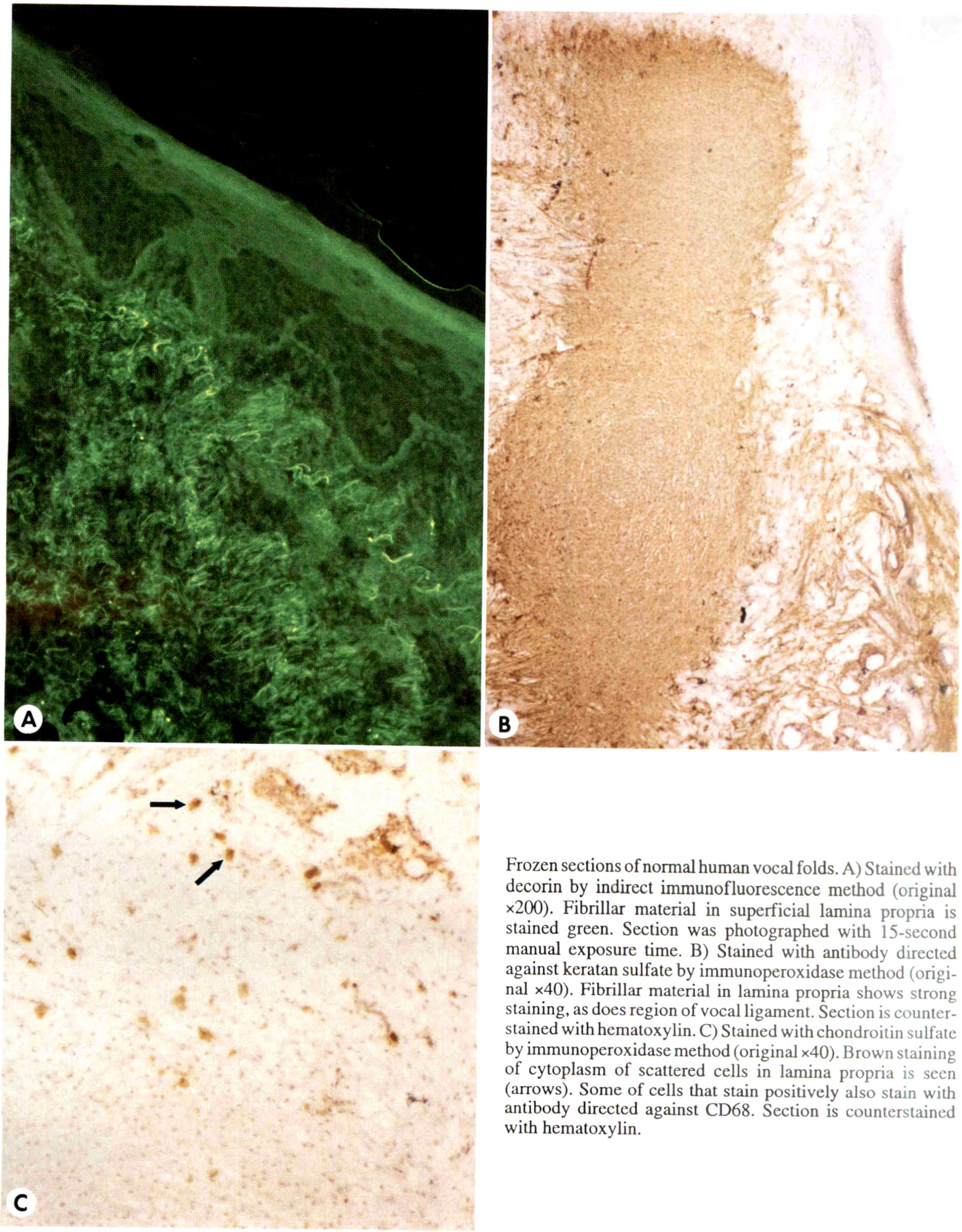
In summary, the fibrillar component of the lamina propria stained with keratan sulfate and decorin; vocal fold ligament stained with keratan sulfate; basement membrane zone stained with heparan sulfate proteoglycan; and cytoplasm of many macrophages and perhaps fibroblasts stained with heparan sulfate proteoglycan, chondroitin sulfate, and hyaluronate receptor (summary of results in Table).

DISCUSSION

The results of this work indicate that diverse proteoglycans are found in the lamina propria and may play an important biologic role, as demonstrated for proteoglycans present in other tissues.

The intense staining of the fibrillar components of the lamina propria and specifically of vocal fold ligament with keratan sulfate suggests an important role for the proteoglycan fibromodulin. The staining pattern for this proteoglycan outlines the vocal ligament area in a way not previously fully appreciated by the authors. Previous descriptions of the vocal ligament have been based on histologic stains of elastin and collagen. The delineation between the superficial tissues and the deeper structures such as the vocal ligament is often not clear histologically. The keratan sulfate stain demarcates an area that appears to correlate with the vocal ligament. Fibromodulin, with its keratan sulfate glycosaminoglycan attachments, has been found to bind collagen types I and II in other tissues. Collagen types I and II also have been localized to lamina propria and are known to be major components of the vocal ligament. The presence of fibromodulin in the extracellular matrix results in delayed collagen fibril formation leading to formation of thinner fibrils and inhibition of collagen matrix contraction.[9-11]

Decorin, a proteoglycan present mainly in the superficial layer of the lamina propria, has functions in common with its family member fibromodulin. It



Frozen sections of normal human vocal folds. A) Stained with decorin by indirect immunofluorescence method (original ×200). Fibrillar material in superficial lamina propria is stained green. Section was photographed with 15-second manual exposure time. B) Stained with antibody directed against keratan sulfate by immunoperoxidase method (original ×40). Fibrillar material in lamina propria shows strong staining, as does region of vocal ligament. Section is counterstained with hematoxylin. C) Stained with chondroitin sulfate by immunoperoxidase method (original ×40). Brown staining of cytoplasm of scattered cells in lamina propria is seen (arrows). Some of cells that stain positively also stain with antibody directed against CD68. Section is counterstained with hematoxylin.

binds to type I and II collagen fibrils and changes the kinetics of collagen fibril formation. This binding is thought to regulate the size and morphology of collagen fibrils. In vitro, the presence of decorin has resulted in thinner and smaller collagen fibrils.[9,11] It is curious that fibromodulin and decorin are closely related proteoglycans, both in function and structure, yet decorin is found predominantly in the superficial layer of vocal fold lamina propria and fibromodulin is intensely present in the intermediate and deep layers of the vocal fold lamina propria. As these findings are correlated with our knowledge of collagen fiber population density in the various lamina propria layers, a biologic and perhaps therapeutic role may be established for these proteoglycans.

Aggrecan does not appear to have an important role in vocal folds, as indicated by our results. Since aggrecan has both chondroitin and keratan sulfate glycosaminoglycans, it would have been expected that the pattern of staining of these glycosaminoglycans would overlap if aggrecan were present. We did not find this congruency of staining. By contrast, versican appears to play a role in the lamina propria of the vocal fold. Its presence is suggested by the finding of abundant cytoplasmic staining of macrophages with antibody against the hyaluronate receptor. Versican is involved in interaction with hyaluronic acid. Versican's structure includes a hyaluronic acid binding domain, and consequently its activity is thought to be related to hyaluronic acid in the extracellular matrix.[12] Hyaluronic acid, which can have voluminous configurations with large, repeating networks of molecular structure, is important in tissue viscosity, osmosis, and flow resistance.[13] These properties may make this proteoglycan very important in tissue oscillation.

Heparan sulfate proteoglycan is found in basement membrane zones and also binds matrix components such as collagen IV, laminin, and the structural glycoprotein fibronectin.[14] Fibronectin is present in normal vocal fold lamina propria, although it has been implicated in benign laryngeal disease.[26] Thus, the importance of interaction of proteoglycans with fibronectin should not be overlooked and needs further research.

The significance of heparan sulfate proteoglycan can be further deduced by examining tissues undergoing fibrosis and scars. The presence of heparan sulfate at these sites during collagen polymerization may result in local changes in the collagen matrices that affect the ability of fibroblasts to remodel the matrix. The discontinuous packing of collagen fibrils, as promoted by heparan sulfate, results in abnormal arrangements of collagen fibrils and decreased tensile strength of scar tissue.[27] The contribution of heparan sulfate proteoglycan must be considered in vocal fold injury.

Chondroitin sulfate, heparan sulfate proteoglycan, and hyaluronate receptor exhibit a common feature of staining the cytoplasm of macrophages and fibroblasts in the lamina propria, as indicated in our research. A study comparing glycosaminoglycan synthesis by human fibroblasts from normal skin, normal scar, and hypertrophic scar suggests the influence of these specific proteoglycans on organization of collagen following vocal fold injury. Hypertrophic scar tissue contains increased amounts of chondroitin sulfate, dermatan sulfate, heparan sulfate, and hyaluronic acid. In addition, the collagen from hypertrophic scar has an altered three-dimensional arrangement compared to that found in normal skin and normal scar. It appears that increased amounts of glycosaminoglycans protect the collagen from normal remodeling by collagenase, leading to the collagen accumulation and excessive scar formation.[28] Therefore, increased synthesis of proteoglycans by fibroblasts secondary to a vocal fold injury may result in scar formation (as evidenced by increase in collagen fibers). This subsequently could lead to dampening of the mucosal wave and result in decreased vocal fold vibration. Assessment of those proteins in benign laryngeal disease is needed.

The cytoplasmic localization of glycosaminoglycans in lamina propria of macrophages of normal vocal folds may suggest a role for these macromolecules in responses to inflammatory stimuli. It has been shown that glycosaminoglycan synthesis is altered in the presence of the cytokine IL-1, a central mediator of inflammation. The IL-1 causes fibroblasts to produce less versican and more hyaluronic acid (see Table, biologic properties of both).[15,29] Such changes could have pronounced effects on vocal fold lamina propria properties. Macrophage chondroitin sulfate production has been shown to be increased by exposure to inflammatory stimuli such as bacterial polysaccharide.[30] Since macrophages can produce IL-1 in response to inflammation, they can directly influence fibroblast production of these glycosaminoglycans as well.

In summary, using monoclonal antibodies, we have demonstrated that decorin and probably fibromodulin have a lamina propria layer specificity. Other proteoglycans are also present in the lamina propria. Alteration in the concentration or production of the proteoglycans could have a profound effect on the physical properties of the extracellular matrix. Our observations establish a baseline for the normal distribution of proteoglycans. Further study will allow precise quantitative analysis of macromolecules, as well as elucidation of specific vocal fold disease states that exhibit distinctive changes in the proteoglycan content and distribution in the lamina propria.

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VISUALIZING THE PEDIATRIC AIRWAY: THREE-DIMENSIONAL MODELING OF ENDOSCOPIC IMAGES

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Three-dimensional reconstruction of medical images has emerged as an important visualization tool for studying complex anatomy. These tools have found important applications in neurology and plastic surgery using computed tomography (CT) and magnetic resonance imaging (MRI) data. However, CT and MRI do not sufficiently delineate lesions of the pediatric airway. Inspection through the rod lens telescope remains the standard diagnostic method. A video recording of an endoscopic procedure is essentially a sequence of two-dimensional images captured as the telescope traverses the airway lumen. Using digitized endoscopic video recordings and computer graphics reconstruction techniques, we have developed a preliminary three-dimensional modeling system for the pediatric airway. A series of normal and abnormal telescopic airway examinations were video recorded. Serial sections were obtained by digitizing the video images at uniform intervals as the scope traversed the airway lumen between the vocal folds and the carina. The digitized images were calibrated and used to reconstruct the airway lumen in three dimensions. Classifying airway abnormalities according to the minimal cross-sectional area or with descriptive terms can be subjective and dependent on the endoscopist's observational skills. We hope that this preliminary work will lead to more precise and understandable methods for representing complex airway lesions.

KEY WORDS — airway, computer graphics, endoscopy, medical imaging.

INTRODUCTION

Airway inspection through the rod lens telescope is the standard method for visualizing the pediatric airway. Ongoing developments in endoscopic optics and illumination have resulted in improved resolution and access to lower airway lesions. The endoscopic image, however, remains essentially two-dimensional (2-D) because of depth-of-field limitations and optical distortion. The experienced endoscopist learns to compensate for optical distortion and develops the habit of mentally reconstructing the three-dimensional (3-D) nature of the airway from sequential 2-D views acquired as the scope moves through the lumen. This is usually sufficient for clinical purposes. However, there are instances in which it can be difficult to understand the morphology of the airway as it changes along its long axis. This can arise, for example, in cases of acquired laryngotracheal stenosis in which clinical grading does not fully characterize changes in the lesion's shape as it is viewed endoscopically along its length.

Concurrent with recent advances in endoscopy and video imaging, developments in computer graphics and image processing have expanded the capabilities of medical imaging into the third dimension. Three-dimensional reconstructions of computed tomography (CT) and magnetic resonance imaging (MRI) are now standard visualization tools in many clinical settings.[1] Using computer image processing in digitized video images and graphical 3-D reconstruction techniques, we have developed an initial 3-D airway modeling system for endoscopic images.

TERMINOLOGY

Image. A 2-D representation of a picture that can be stored and manipulated in computer memory as a set of pixels (picture elements); pixels are characterized by their location on the picture plane (x and y coordinates) and their color (pixel value).

Slice. A 2-D image representing a planar cross section of a structure.

Segment. To digitally separate portions of an image representing a certain tissue type; used to define the airway lumen edges in two dimensions.

Model. A 3-D computer representation of a surface built from a set of coordinates in space; used to represent the lumen of the airway.

Render. To display a 3-D model on the computer terminal screen.

Resolution. The minimum distance between two separately discernible lines or points in an image.

Planar Distortion. Aberration in the appearance of parts of an image due to the shape of the viewing lens

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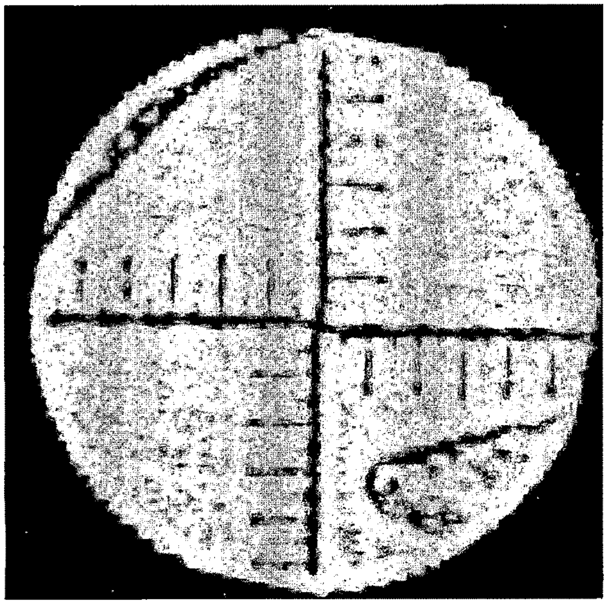

Fig 1. Digital image of glass reticle micrometer ruled in 0.1-mm markings. Used to develop scaling correction factors for endoscopic images.

(lens distortion) or the distance of the object from the lens (perspective distortion).

Scale Factor. Number applied to image or model coordinates to account for planar distortion (x-y scale factor) or slice thickness (z scale factor).

MATERIALS AND METHODS

Image Calibration. Planar (x and y axis) calibration factors were compiled from images of a contact glass slide micrometer by using the Karl Storz 27018A telescope and imaging system employed during endoscopy (Fig 1). The micrometer was ruled in 0.1-mm markings. A constant distance from the end of the telescope to the image plane was maintained by using a marked ureteral catheter passed through the suction channel of the bronchoscope ventilating tube. From these measurements, a table of calibration factors based on the distance of the lumen edge from the lumen center was assembled. This was implemented as a lookup table in the modeling program.

KARL STORZ 27018A TELESCOPE PARAMETERS

Working Distance (mm)*	*Field Diameter (mm)*	*Resolution (mm)*	*Maximum Distortion*	*x-y Scale Factor*
3	5.8	0.1	0.91	1.00-1.10
5	7.8	0.1-0.5	0.88	1.00-1.14
7	11.1	0.1-0.5	0.83	1.00-1.20
9	12.1	0.1-0.5	0.80	1.00-1.25
11	13.4	0.5	0.75	1.00-1.33
13	14.5	0.5-1.0	0.75	1.00-1.33
15	15.8	1.0	0.83	1.00-1.20

*Three millimeters was minimum working distance for 27018A telescope. This was distance from tip of telescope to end of ventilating sheath. Beyond working distance of 15 mm, images were out of focus (resolution worse than 1.0 mm).

The distance from the carina to the vocal folds was used as a reference value to calibrate the model along the long (z) axis. Each planar slice was assigned a thickness equal to the sampling distance. The z coordinates of the model were then scaled by the number of pixels equal to this distance as measured during the calibration imaging (Fig 2). The scaling factors are summarized in the Table.

Three-dimensional Reconstructions. A series of four airway endoscopies in children ages 8 months to 11 years were videotaped through the Karl Storz 27018A (2.2 mm) 0° telescope and video camera system. Digital images were captured from the video recordings. Serial images were captured at uniform interval distances from the carina as the scope traversed

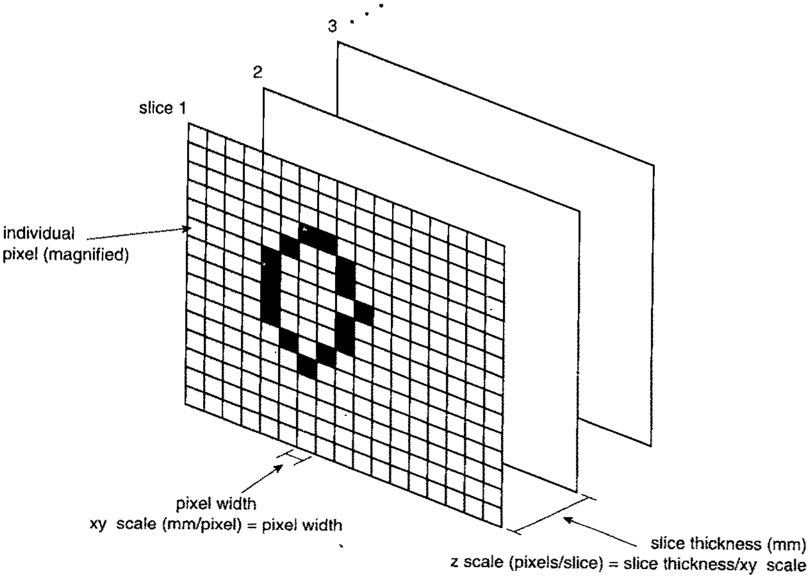
3 . . .
2
slice 1
individual pixel (magnified)
pixel width
xy scale (mm/pixel) = pixel width
slice thickness (mm)
z scale (pixels/slice) = slice thickness/xy scale

Fig 2. Calibration and scaling process.

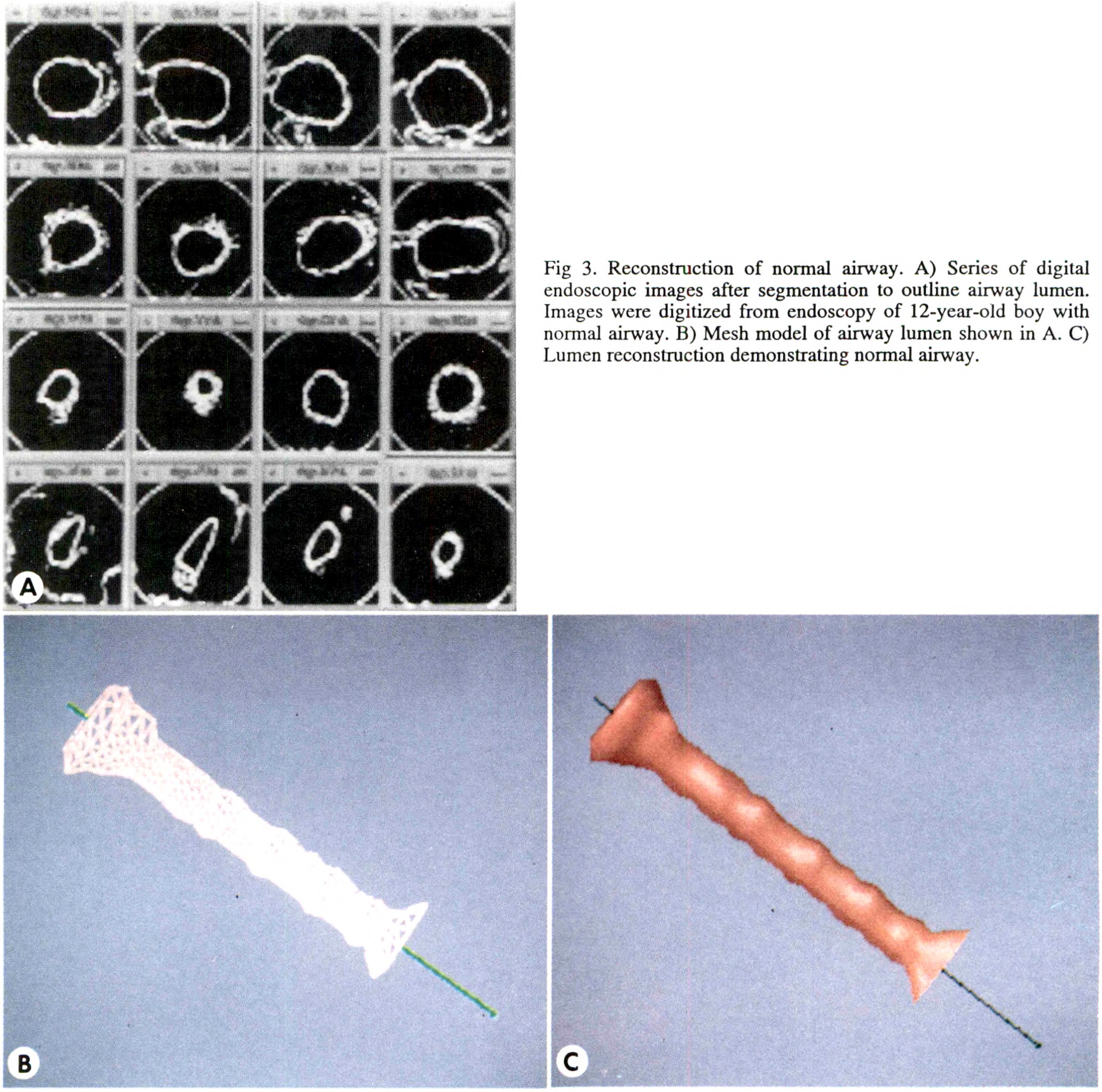

Fig 3. Reconstruction of normal airway. A) Series of digital endoscopic images after segmentation to outline airway lumen. Images were digitized from endoscopy of 12-year-old boy with normal airway. B) Mesh model of airway lumen shown in A. C) Lumen reconstruction demonstrating normal airway.

the airway. Magnification was constant throughout the calibration and endoscopic imaging.

The captured serial images were digitally processed to outline the airway lumen (Fig 3A). Higher-contrast images were segmented by using an automatic edge detection algorithm.[2] The sections of stenotic segments often lacked sufficient contrast for automatic edge detection and had to be outlined manually. The x and y polygonal coordinates for each lumen slice were scaled by the appropriate entry in the calibration table. The resulting set of planar lumen contours were used to construct a geometric mesh model of each airway (Fig 3B). The z coordinates of the mesh model were scaled by the number of pixels equal to the slice thickness as measured during calibration. After calibration, the mesh model was rendered to scale by using computerized rendering techniques for geometric models (Fig 3C).

The image processing, modeling, calibration, and rendering software modules (*Endos*) were developed by the first author in the C/C++ programming language. Each module was implemented on a Silicon Graphics Indigo-2 Extreme computer with digital image capturing hardware. The program runs under the UNIX operating environment with the X-Window system. The rendering pipeline is summarized in Fig 4.

RESULTS

Calibration. Our approach to calibrating the images was designed to avoid applying a single calibra-

Endoscopy Recording
CCD camera
VCR

↓

Serial Image Digitization
Frame capture hardware
Imaging software

↓

Image Segmentation (Edge Detection)
Graphics computer workstation
Image processing software

↓

Three Dimensional Wire Frame Model
Graphics computer workstation
Endos software

↓

Three Dimensional Rendered Model
Graphics computer workstation
Endos software

Fig 4. Image acquisition and rendering pipeline.

tion factor to all parts of the image when it is known that distortion changes from the center to the periphery of the image. Distortions in the planar images include x-y planar distortion due to the lens and perspective distortion. We used line segments in the calibration image measured in pixels. For example, a 0.5-mm line in the center of the image measured 5 pixels for the 27018A scope at a 9-mm working distance. This was used as the undistorted conversion factor (pixels per millimeter) when converting between measured distances and numbers of pixels. The cumulative number of pixels was calculated along successive 0.5-mm intervals moving from the center to the periphery of the calibration image. Using the cumulative pixel value and the central measurement, we calculated a set of distortion factors for each possible x or y coordinate value.

$$distortion_{\text{num pixels}} = \frac{calibration\ measurement\ (mm) \times conversion\ factor\ (pixels/mm)}{number\ of\ pixels\ (pixels)}$$

The x-y scale factor for a given pixel value from center is the inverse of the distortion at that pixel.

$$x\text{-}y\ scale = \frac{1}{distortion}$$

During modeling, each vertex x and y coordinate was corrected by the x-y scale factor for that particular coordinate value. Since the reconstructed models consisted of up to 40,000 vertices (80,000 x-y coordinates), the scale correction factors had to be applied automatically as part of the modeling algorithm. This was implemented as a lookup table in which each vertex x-y scale factor was held in computer memory. The set of x-y scale factors was specific for the scope and the working distance.

Assembling a calibration table for a given scope and working distance corrected for planar and perspective distortion. The amount of distortion varied with the working distance between the end of the lens and the object. As the working distance increased, the field of view diameter and the degree of distortion increased; the resolution decreased. Using the scope at a working distance of 9 mm yielded a sharply focused image with approximately a 10% distortion

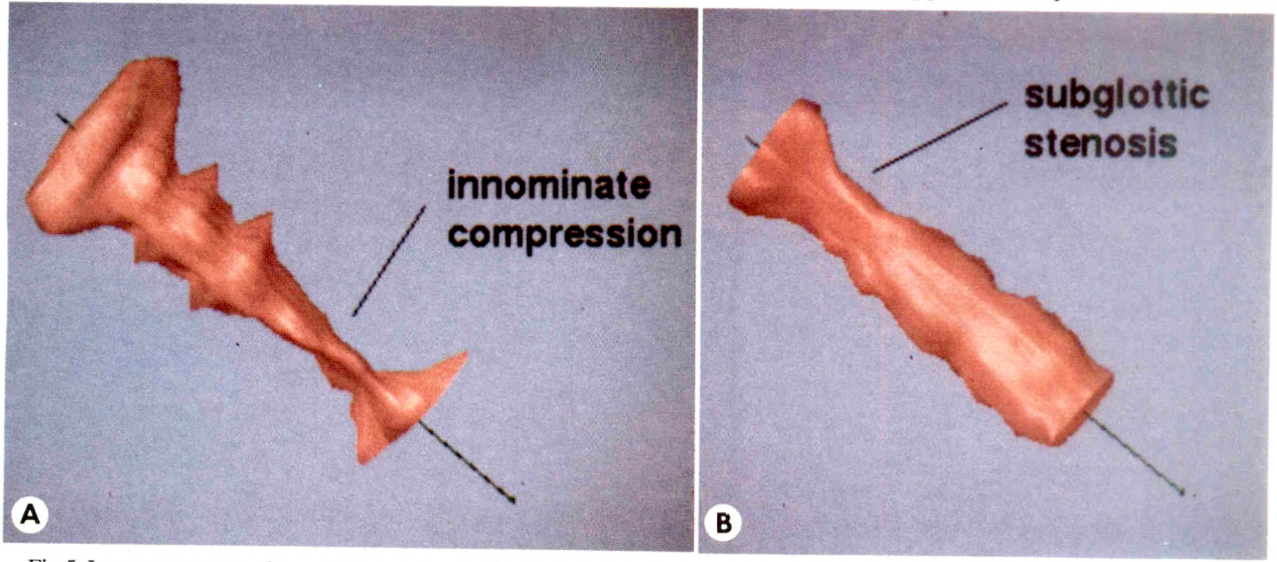

Fig 5. Lumen reconstructions. A) Demonstrating innominate artery compression. Patient is 4-month-old boy with severe stridor and failure to thrive. B) Demonstrating subglottic stenosis. Patient is 11-year-old boy with acquired subglottic stenosis (Cotton grade 2).

in the periphery. Entries in the x-y scale factor table ranged from 1.00 to 1.25, and the overall resolution for the system was between 0.1 and 0.5 mm. Image distortion parameters as a function of working distance are summarized in the Table.

Scaling along the long axis of the airway (z) is more problematic. We found that exact distances along this axis were difficult to measure during endoscopy. However, we felt that it was sufficient to measure the total length of the airway from the carina to the glottis and then record the airway as the scope was slowly and uniformly withdrawn. The individual planar slices were then digitized at uniform intervals. Using the conversion factor (pixels per millimeter) from the x-y calibration image, we calculated the thickness of each slice.

$$\text{slice thickness} = \frac{\text{lumen length (mm)} \times \text{conversion factor (pixels/mm)}}{\text{number of slices}}$$

The slice thickness became the z scale factor in the modeling algorithm and was assigned for each image set.

Three-dimensional Images. Reconstructions using 10 to 20 slices adequately depicted upper airway morphology in cases in which the endoscopist noted a normal or uniformly deformed airway. More complex deformities required a greater number of sections (50 to 100) to yield 3-D images consistent with the surgeon's impression acquired during endoscopy (Fig 5).

DISCUSSION

Digital Imaging and Visualization. Images displayed on a computer terminal screen can be captured from real-world data (eg, scanning and video capture) or generated algorithmically within the computer's central processing unit (eg, modeling). Most 2-D medical images are captured by scanning living tissues and measuring some tissue parameter such as x-ray attenuation or nuclear magnetic resonance.[3] The computer stores scanned images in digital form as a rectangular array of numbers in memory called the frame buffer. As the frame buffer is read continuously at approximately 70 Hz, its contents are translated into corresponding screen pixel values.

Endoscopic video imaging operates similarly. The digital images are captured from analog video signals by means of an analog-to-digital converter, stored in the frame buffer, and displayed.

Three-dimensional reconstruction of medical images has emerged as an important visualization tool for studying complex morphology. Current applications include 3-D reconstruction of CT and MRI data in craniofacial surgery,[4] orthopedics,[5] and neurosurgery.[6] Three-dimensional reconstructions from ultrasound data have been applied in obstetrics[7] and vascular surgery.[8]

A number of algorithms have been described to reconstruct 3-D images from 2-D scanned data.[9-11] The method used here is a variation of the skimming algorithm for reconstructing hollow objects.[12] Each algorithm generates a scaled 3-D model of the object from a set of its 2-D cross sections. The accuracy of the reconstructed model is a function of the number and thickness of the cross sections and the accuracy of the scaling calculations.

Resolution and Calibration. Video imaging and recording have been recognized as important adjuncts to airway endoscopy, particularly for documentation and teaching. Often unknown to the user, optical and video technology can introduce errors through resolution reduction and spatial distortion.[13] During the design of the modeling algorithm, it was necessary to consider the resolution and spatial distortion issues separately.

Image spatial resolution is defined as the minimum distance between two separately discernible points on the image. Ultimately, the final image resolution is the coarsest of the optical, video, and digital resolutions within the system. Optical resolution in an endoscopic system is a function of the lens system used in the telescope. The Karl Storz telescopic system used in this study achieves an optical resolution of at least 0.03 mm. The manufacturer would not release optical bench measurements for proprietary reasons. Our current video imaging system operates at 440 lines of resolution; at standard magnification, approximately 250 horizontal lines cover the image area. The spatial resolution of the digital image is also a function of pixel size. Our imaging system operates at a resolution of 1,280 × 1,024 pixels. The video horizontal line resolution is the limiting factor in the overall system resolution. The overall resolution was 0.1 to 0.5 mm.

The error in the model coordinate calculations introduced by resolution reduction can be estimated as twice the overall resolution divided by the average dimensions of the reconstructed lumen. A calculated error of 0.3% to 1.3% compares favorably with our ability to measure the cross-sectional area of stenotic segments with standard endotracheal tubes. The external diameter change from one tube size to the next is 5 mm. It certainly compares favorably with simple subjective endoscopic impressions of the airway caliber.

Spatial distortion can introduce errors in the modeling process because of the optical wide-angle effect in the lens system. Stated geometrically, two points on the image that are far from the center are resolved differently than two similar points that are near the center. The overall effect is to spatially compress the image at the periphery. The design and engineering of the rod lens optical system accepts this distortion in order to achieve an acceptable field of view. Scale correction is intended to uncompress the distorted parts of the image. The model coordinate scaling factors increase as the coordinates move further from the image center. The scaling operation maintained model proportionality regardless of the scope magnification or image field size. Since the imaging system magnification is kept constant during calibration and endoscopy, measurements taken directly from the model or 2-D images are accurate to within the system's overall resolution.

The modeling process described herein required relatively few points on each 2-D image when compared to the total number of pixels in each image (several hundred compared to several hundred thousand). Therefore, rather than try to apply a single calibrating function to each entire slice, we empirically calibrated the points of interest on each slice edge by comparing each point (pixel) coordinate pair with a corresponding entry in a calibration table and then applying the scaling factor to the coordinates. This is performed in software prior to building the mesh model. Since the calibration table was derived from previously measured images, the micrometer readings, no significant error was introduced during the calibration process.

Three-dimensional Visualization of Acquired Laryngotracheal Stenosis. The airway deformity associated with laryngotracheal stenosis (LTS) is often irregular and has a complex shape when viewed three-dimensionally. Laryngotracheal stenosis is diagnosed and measured endoscopically. A number of systems have been proposed to classify LTS in terms of the cross-sectional severity. Perhaps the most popular system grades the stenosis as a function of the child's size and the largest standard endotracheal tube admissible through the stenotic airway.[14]

Classifying LTS according to its minimal cross section does not fully describe the clinically relevant characteristics of the lesion, including the shape and length. These factors remain descriptive and somewhat subjective. They are at best dependent on the endoscopist's observational skills and ability to fully describe the endoscopic findings. These limitations become more apparent when evaluating a series of observations taken from the same lesion over time or when planning open surgical correction. Accurate assessment for determining graft dimensions and placement requires a complete 3-D understanding of the lesion. Three-dimensional visualization of endoscopic images could assist in airway management, just as CT and MRI visualization techniques assist in the assessment and management of craniofacial and central nervous system lesions.

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SUPRAGLOTTIC LARYNGECTOMY: FUNCTIONAL AND ONCOLOGIC RESULTS

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Between January 1980 and December 1989, 110 patients with squamous cell carcinoma of the supraglottis were treated with supraglottic laryngectomy and neck dissection. The stage distribution was stage I 23%, II 34%, III 15%, and IV 28%. Adjuvant radiotherapy (5,000 to 6,500 cGy) was given to all pN+ cases. All patients were followed until death or for a minimum of 36 months, with an average of 65 months. Decannulation was achieved in 96% of the cases, with only 1 patient undergoing total laryngectomy because of aspiration. The average hospital stay was 22 days. Arytenoid edema was a frequent cause of delayed decannulation in patients undergoing radiotherapy. The overall 3-year survival was 78%, with 10 patients dying of unrelated causes. Local control was 94.6% and regional control was 83.6%. No significant difference was found in survival according to T stage, but survival rate was significantly influenced by N stage.

KEY WORDS — aspiration, functional results, supraglottic laryngectomy, survival.

The options for the treatment of supraglottic carcinoma include surgery, radiotherapy, or a combination of both. The selection of treatment depends on the patient evaluation of the sequelae against the chances for cure. The information given to approach this decision will heavily depend on the experience, infrastructure, and philosophy of the responsible physician and the institution.

In our practice, surgery is the preferred option for the treatment of supraglottic carcinomas. For embryological and anatomic reasons,[1,2] supraglottic laryngectomy allows the removal of selected supraglottic tumors, preserving a functioning larynx and avoiding a permanent tracheostomy. The oncologic results of supraglottic laryngectomy are similar to those of total laryngectomy.[3,4]

The purpose of this study is to update our experience with supraglottic laryngectomy and to review the functional and oncologic results of the operation.

MATERIALS AND METHODS

From January 1, 1980, to December 31, 1989, 110 previously untreated patients with squamous cell carcinoma of the supraglottis were treated at the Departments of Otorhinolaryngology of Juan Canalejo (61) and La Paz (49) Hospitals. All patients were male, with a mean age of 58 years (range, 36 to 74). Only 17% of the patients were over 65 years of age.

All patients underwent surgical treatment with horizontal supraglottic laryngectomy[5] for the primary disease. No extended procedures beyond the circumvallate papillae or including the arytenoid were performed. Unilateral neck dissection was performed in 68 patients (62%), bilateral neck dissection in 36 patients (33%), and no neck dissection in 6 patients (5%). A complete standard functional neck dissection (FND)[6] was performed in 131 cases, and the remaining 9 dissections were classic procedures. No selective operations were done in this series. Pathologic staging according to the 1987 UICC classification was used for the primary tumor and the neck (Table 1). Postoperative radiotherapy was used in 42 patients (38%). Doses ranged from 5,000 to 6,500 cGy in a 200 cGy/d, 5 d/wk schedule. Radiotherapy was delivered through an anterior field and two lateral opposed fields, beginning 6 weeks or less after neck dissection and before recurrence. Indications for postoperative radiotherapy included positive margins and node metastases. The tracheostomy was left open with a fenestrated corked tube in those patients undergoing postoperative radiotherapy. Swallowing training began 6 to 8 days after surgery. The feeding tube was not removed until the patient was able to swallow without significant aspiration.

Follow-up data were obtained for all patients until the time of death or until December 31, 1992. No patient was excluded from evaluation. The data were collected from the head and neck cancer registry database and analyzed for postoperative hospitaliza-

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TABLE 1. NODAL STATUS BY STAGE OF PRIMARY TUMOR

	N0		*N1*		*N2*		*N3**		
Stage	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*% Positive Nodes*
T1	25	22.7	5	4.5	3	2.7	4	3.6	33
T2	37	33.6	6	5.5	8	7.3	4	3.6	32
T3	4	3.6	2	1.8	0	0	2	1.8	50
T4	6	5.5	2	1.8	1	0.9	1	0.9	40

*N3 stage includes both single nodes of more than 6 cm in greatest diameter and nodal masses larger than 6 cm in which no individual node could be histologically separated.

tion, complications, cause of death, site of recurrence, treatment of recurrence, and survival. A Kaplan-Meier analysis was performed to obtain survival data.

RESULTS

Local Control. Local control 3 years after surgery was 94.6%. The analysis of local recurrence and outcome is shown in Table 2. The average time for recurrence was 15 months, and the laryngeal remnant was the most frequent site of recurrence (4/6). One patient with a synchronous glottic and lung carcinoma 80 months after supraglottic laryngectomy was not considered to have a recurrence. The 3-year actuarial survival according to T stage was 75% for T1, 78% for T2, 87% for T3, and 80% for T4. No significant difference was found.

Neck Recurrences. Eighteen patients (16.4%) had recurrences in the neck. Table 3 summarizes the neck control with reference to the primary treatment, salvage procedure, and outcome. The recurrence rate in N0 patients was 11% (8/72), and that in N+ patients was 26.3% (10/38). All N+ patients underwent postoperative radiotherapy. In 78% of the cases (14/18) the neck recurrence developed within 18 months after treatment. Fifteen of these patients (83.3%) died irrespective of salvage treatment. The 3-year actuarial survival was 87% for N0, 86% for N1, 58% for N2, and 27% for N3 ($p < .05$; Fig 1).

Survival. The overall 3-year actuarial survival was 78%. Five years after the operation, 72.6% of the patients were still alive (Fig 2). Ten patients died of unrelated causes with no evidence of disease.

Hospital Stay. Postoperative hospitalization ranged from 11 to 91 days (average, 22 days). In 52% of the patients (57/110) the hospital stay was less than 20 days, and in 16% (18/110) hospitalization was longer than 25 days. Patients staying more than 35 days had in most cases (72%; 13/18) wound complications such as delayed healing, wound sloughing, fistula, or pulmonary complications due to aspiration (17%; 3/18). One patient was discharged from the hospital 91 days after initial surgery because of continuous aspiration that required total laryngectomy.

Postoperative Complications. All our patients had swallowing problems once they began to swallow. However, in most cases they were able to feed themselves within 10 days postoperatively. The feeding tube was removed between 11 and 15 days after surgery in 7 patients (6%), between 16 and 20 days in 50 patients (45%), between 21 and 25 days in 35 patients (32%), between 26 and 35 days in 10 patients (9%), and after 35 days in 8 patients (7%). One year after surgery, 49% of the patients had no problems with aspiration, 42% had occasional minor aspiration when drinking liquids, and 8% had frequent aspiration problems. None of our patients had pulmonary infections or significant weight loss. One patient underwent a total laryngectomy 3 months after surgery because of permanent aspiration.

Decannulation was possible in 94.5% of the patients (104/110). Three patients were not decannulated because of laryngeal stenosis, and 3 because of arytenoid edema. Edema of the arytenoid during radiotherapy required the reopening of the tracheostomy in 2 patients. In 5 patients direct laryngoscopy was necessary to remove the redundant arytenoid edema-

TABLE 2. ANALYSIS OF LOCAL RECURRENCES

TNM	*Time Since Surgery (mo)*	*Site of Recurrence*	*Neck Dissection*	*Postop Radiotherapy*	*Outcome*
T4N0M0	9	Larynx	Unilateral	No	Died with local disease; refused TL
T2N0M0	13	Larynx	Unilateral	No	Free of disease 49 months after TL
T1N3M0	13	Tongue base and neck	Unilateral	Yes	Died with local and regional disease
T2N3M0	13	Larynx	Bilateral	Yes	Died with local and distant disease
T1N0M0	17	Tongue base and neck	Bilateral	No	Died with local and regional disease
T1N0M0	24	Larynx	Unilateral	No	Free of disease 64 months after TL

TL — total laryngectomy.

TABLE 3. ANALYSIS OF NECK CONTROL AFTER INITIAL TREATMENT

TNM	*Time Since Surgery (mo)*	*Site of Recurrence**	*Neck Dissection†*	*Postop Radiotherapy*	*Treatment*	*Outcome*
T4N0M0	18	Ipsilateral	Unilateral	No	Neck dissection	Free of disease
T2N0M0	18	Contralateral	Unilateral	No	Neck dissection and radiotherapy	Free of disease
T2N0M0	9	Contralateral	Unilateral	No	Neck dissection	Free of disease
T4N0M0	6	Ipsilateral	Unilateral	No	Neck dissection	Died of neck recurrence
T2N3M0	9	Ipsilateral	Unilateral	Yes	Refused treatment	Died of neck recurrence
T2N0M0	15	Ipsilateral	Unilateral	No	Neck dissection	Died of neck recurrence
T1N0M0	48	Ipsilateral	Bilateral	No	Refused treatment	Died of neck recurrence
T3N3M0	5	Ipsilateral	Bilateral	Yes	Palliative chemotherapy	Died of neck recurrence and esophageal cancer
T2N2M0	11	Ipsilateral	Bilateral	Yes	Palliative chemotherapy	Died of neck recurrence
T2N2M0	15	Ipsilateral	Unilateral	Yes	Palliative chemotherapy	Died of neck recurrence
T1N2M0	18	Contralateral	Unilateral	Yes	Palliative chemotherapy	Died of lung metastases
T2N1M0	35	Contralateral	Unilateral	Yes	Neck dissection	Died of neck recurrence
T3N1M0	28	Contralateral	Unilateral	Yes	Palliative chemotherapy	Died of neck recurrence
T2N0M0	20	Contralateral	Unilateral	No	Refused treatment	Died of neck recurrence
T1N3M0	11	Ipsilateral	Bilateral	Yes	Palliative chemotherapy	Died of neck recurrence
T2N3M0	10	Contralateral	Unilateral	Yes	Palliative chemotherapy	Died of neck recurrence
T1N3M0	13	Contralateral	Unilateral	Yes	Neck dissection	Died of neck recurrence
T1N0M0	17	Contralateral	Unilateral	No	Total laryngectomy, neck dissection, and radiotherapy	Died of neck recurrence

*Ipsilateral — recurred in dissected side of neck, contralateral — recurred in nondissected side of neck.

†Done at time of initial surgery.

tous mucosa to obtain a safe glottic airway. Other surgically related complications are shown in Table 4.

DISCUSSION

The treatment of supraglottic carcinoma is a controversial, unsettled issue.[7-9] The debate is established beween conventional surgery and radiotherapy, with laser surgery asking for a place in the discussion. Each option has its functional and oncologic consequences. The patient must know in advance the rate of survival, complications, time of recovery, and functional sequelae associated with each treatment option in order to make a choice.

Local control of supraglottic carcinomas treated with supraglottic laryngectomy ranges between 95% and 100%.[3,7-9] This is similar to the control rate of total laryngectomy. It is difficult to compare results from different series treated in different institutions by different methods. Bias is a sure defect. Bearing this in mind, our results are comparable to those reported in other surgical series.[7-9] Reports comparing series treated in the same institution show better local control with surgery than with radiotherapy.[9,10] On the other hand, very little difference in survival is found between different treatment programs, namely, surgery alone, combined treatment, or irradiation for cure with surgery for salvage. Programs using supraglottic laryngectomy end up with more patients with their glottic voice preserved. Those patients with a local recurrence after radiotherapy will lose their

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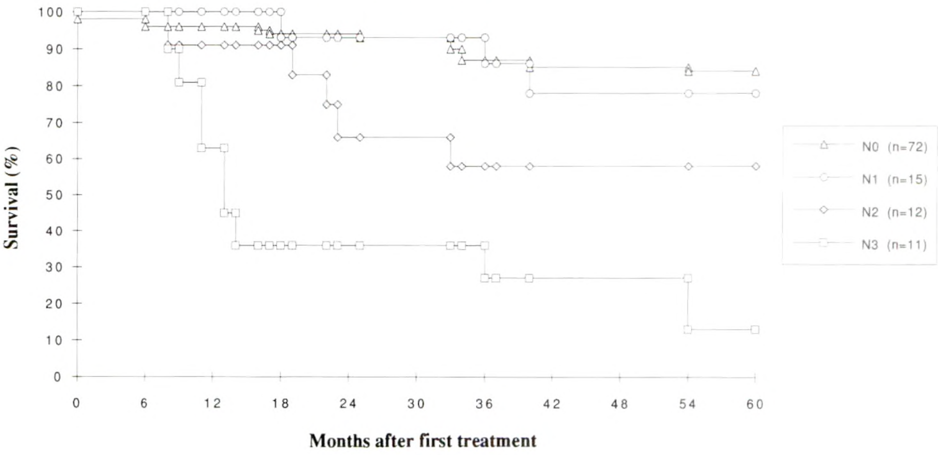

Fig 1. Actuarial 5-year survival according to N stage.

voice at salvage laryngectomy. Supraglottic laryngectomy is not widely used as a salvage procedure.[11] In a report from the University of Florida on 54 patients with lesions anatomically suitable for supraglottic laryngectomy, treated with radiotherapy, overall local control was 75.9%, with 100%, 85%, 50%, and 25% for T1, T2, T3, and T4 lesions, respectively.[12]

Adequate clinical tumor examination to rule out anterior commissure involvement or extension to the vocal cords or arytenoids is critical for patient selection. However, the final decision about whether to perform a supraglottic laryngectomy or not is made after frozen section examination of the surgical margins. Margins should be taken from the patient, not from the surgical specimen. When positive margins are found, a total or near-total laryngectomy should be performed. Relying on radiotherapy for incomplete tumor removal is a risky oncologic concept.

When treating patients with supraglottic carcinoma, the surgeon must keep in mind that survival depends more on regional than on local control. The battle against supraglottic cancer is won or lost in the neck, with the N stage being the single most important prognostic factor. Unfortunately, not only is there no agreement concerning the optimal approach to the neck, but surgery and radiotherapy are frequently combined, with uncertain results. This is especially true when the surgical approach includes FND. The linkage between FND and postoperative irradiation is considered a rule of thumb of oncologic practice by many. However, there is no evidence in the literature of fewer patients dying of cancer after combined therapy in comparison with patients treated with surgery alone. On the contrary, postoperative irradiation increases the rate of complications after supraglottic laryngectomy without extra benefit.

The patients treated with FND in this series represent the last group of patients undergoing routine postoperative irradiation for positive nodes in our practice. Between 1968 and 1989, postoperative radiotherapy was delivered to all our patients with positive nodes after FND. However, we could not find evidence of improved survival.[13] The results of the present study reinforce this impression. The rate of regional failures in patients with positive nodes — all of them undergoing postoperative irradiation — is comparable to the 2-year neck failure rates of N1 and N2 patients treated with neck dissection alone by DeSanto et al.[14] Because of the dubious value of postoperative irradiation for N+ patients in terms of

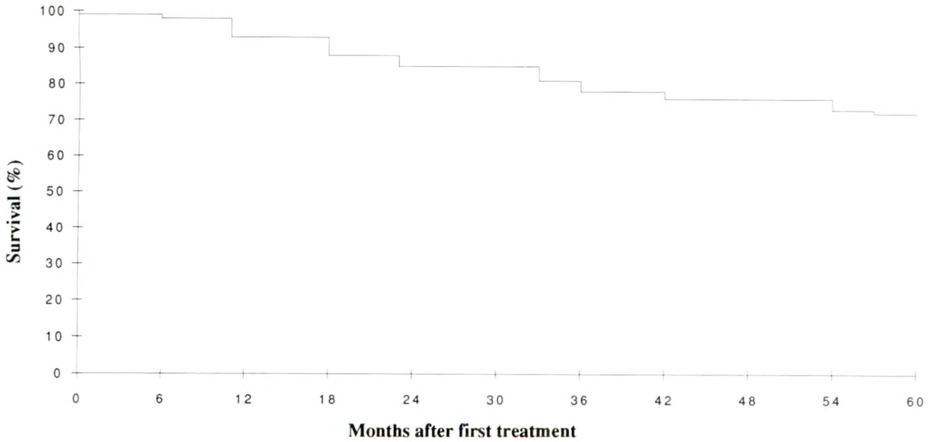

Fig 2. Overall 5-year survival after initial treatment.

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TABLE 4. POSTOPERATIVE COMPLICATIONS

Complication	*No.*	*%*
None	76	69
Seroma	9	8
Delayed wound healing	8	7
Pulmonary complications	6	5
Pharyngocutaneous fistula	6	5
Hemorrhage	3	3
Wound abscess	1	1
Death	1*	1

*Death related to acute and massive hemorrhage.

increased survival, in 1990 we abandoned combined therapy as a routine. The results of our first group of N+ patients treated with surgery alone will soon be published, but no difference in survival has been detected between patients treated with combined therapy and those undergoing surgery alone. This has also been reported by others.[10,15]

Although aspiration is the most frequent postoperative complication, most patients are able to eat satisfactorily within 2 or 3 weeks postoperatively. To improve swallowing, the patient is asked to flex the head until the chin touches the chest. Aspiration is probably the result of the combined effects of more than one single factor. Several procedures have been proposed to facilitate swallowing and avoid aspiration. The effects on swallowing of superior laryngeal nerve preservation, cricopharyngeal myotomy, and hyoid bone sparing are to be proved.[16] On the other hand, removing one arytenoid, the pyriform sinus, or the tongue base is associated with increased aspiration and delayed rehabilitation.[16,17] We never use extended procedures. Tumors requiring the so-called "extended supraglottic laryngectomy" are treated with near-total laryngectomy or total laryngectomy with tracheoesophageal puncture. In our opinion it is better not to perform a supraglottic laryngectomy than it is to convert it to a total laryngectomy because of aspiration.

The devastating effects of aspiration should be contemplated when selecting patients for supraglottic laryngectomy. Age and pulmonary conditions must be carefully evaluated, but should not be used with a rigid mentality. A sturdy 75-year-old patient may be a better candidate than a frail 45-year-old patient. It is a matter of biologic rather than chronologic age.

Airway obstruction retards decannulation. It may be due either to arytenoid edema or to glottic stenosis. To avoid delayed decannulation, all tissue from the supraglottis must be removed during the operation, and no attempt should be made to cover the arytenoids with mucosal flaps. Steroids and endoscopic removal are sometimes needed. To avoid complications in patients receiving postoperative radiotherapy, the tracheostomy should be maintained until the end of radiotherapy.

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RESPIRATORY MANIFESTATIONS OF GASTROESOPHAGEAL REFLUX DISEASE IN PEDIATRIC PATIENTS

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Respiratory manifestations of gastroesophageal reflux disease (GERD) are being recognized with increasing frequency. We present the evaluation and management of four infants and children with unusual respiratory symptoms attributed to GERD. The advantages and disadvantages of diagnostic studies of GERD are discussed, and an evaluation and treatment protocol is presented. Treatment must be tailored to the nature and severity of the patient's presenting symptoms and includes conservative, pharmacologic, and/or surgical management.

KEY WORDS — gastroesophageal reflux disease, larynx, pediatric patient, respiration.

INTRODUCTION

Gastroesophageal reflux (GER) is a common physiologic phenomenon in infants and toddlers. Often described as "spitting up," GER usually resolves spontaneously by 12 to 18 months of age. When the frequency or duration of GER is severe enough to induce symptoms or histologic changes of chronic inflammation, GER becomes pathologic and is termed gastroesophageal reflux disease (GERD).

The incidence of GERD in pediatric patients has increased over the past several decades and reflects improved diagnosis of GER and heightened physician awareness of GER-induced findings. Diagnostic studies available to detect GERD in pediatric patients include extended distal esophageal pH monitoring, barium esophagography with video swallow, technetium scintigraphy, esophageal manometry, endoscopy with or without esophageal biopsy, bronchoalveolar lavage for lipid-laden macrophages (LLMs), and the modified Bernstein or esophageal acidification test.

The manifestations of GERD are diverse and may involve the gastrointestinal (GI), neurobehavioral, and respiratory systems. The GERD-induced GI symptoms are most common and include vomiting, failure to thrive, dysphagia, hematemesis, and esophagitis. Neurobehavioral symptoms are uncommon and include unusual posturing and irritability. Of particular interest to otolaryngologists are GERD-induced respiratory symptoms. These symptoms, like central or obstructive apnea, stridor, recurrent pneumonia, bronchitis, asthma, and chronic cough, may be a direct consequence of aspiration or may be reflex-mediated responses due to stimulation of esophageal or laryngeal sensory receptors. One should consider GERD as a cause or exacerbating factor when typical treatment of the more common causes of these diseases does not effect resolution.

If GERD is diagnosed, treatment must be individualized and based on the general health of the child and the severity of disease. Mild GERD generally is managed conservatively by positional therapy and/or dietary modifications, moderate to severe GERD warrants pharmacologic intervention, and life-threatening GERD necessitates surgical therapy.

This paper reviews the pathophysiology, incidence, symptoms, diagnosis, and management of GERD with a focus on laryngeal and respiratory manifestations. Case studies illustrate the difficulties and delay sometimes encountered in achieving the diagnosis. Appropriate therapy may effect prompt resolution of symptoms.

CASE REPORTS

Case 1. An 8-month-old boy, in the 5th percentile for weight and height, was admitted for evaluation of failure to thrive. Although in the 25th and 90th percentiles for weight and height at birth, frequent prandial and postprandial emesis prevented weight gain. The infant was "congested since birth," developed a chronic, nonproductive cough, and required hospitalization at 7 months of age for respiratory

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distress.

Physical examination confirmed the delayed development in an irritable, thin infant with a hoarse cry. The posterior glottis and vocal folds were erythematous and edematous. Expiratory wheezing and harsh upper airway sounds were evident on auscultation, and a right upper lobe infiltrate was noted on chest radiography.

Diagnostic barium esophagography and video swallow demonstrated frank aspiration on swallowing and an inconsistent cough reflex. Despite the absence of reflux events on the barium study, GERD was suspected and confirmed by extended distal esophageal pH probe monitoring.

Nasogastric feedings, conservative antireflux measures, and ranitidine, an oral histamine type 2 receptor antagonist (H2 receptor antagonist), were initiated. Wheezing, cough, and harsh upper airway sounds resolved nearly immediately, and a repeat esophagogram obtained 2 weeks later showed resolution of the primary (swallow) aspiration. During the 3-week hospital admission, the infant gained 1.2 kg and advanced 2 months in development. The impressive neurologic and nutritional improvements achieved with antireflux therapy alone were sustained, and the infant has remained symptom-free for over 8 months.

This case demonstrates GI and respiratory manifestations of GERD and the delay that often precedes an infant's referral for evaluation. The infant's symptoms were attributed to aspiration, which was confirmed on the barium esophagogram with video swallow. Although the aspiration occurred on swallowing (primary) and in the absence of a reflux event, the prompt resolution of symptoms with antireflux treatment indicts GERD as the inciting event. Primary (swallow) aspiration due to GERD likely occurs through one of two mechanisms. The GERD may induce esophageal motor abnormalities such as abnormal peristalsis and simultaneous contractions[1] that may lead to a dyscoordinated swallow. Alternatively, GERD may initiate a vicious reflux-malnutrition cycle in which severe malnutrition reduces neuromuscular control and promotes further reflux events.

The value of short-term nasogastric feedings in treating GERD was demonstrated in this infant's management. A 7- to 10-day course of continuous infusions may improve nutritional status, halt vomiting, and effect complete resolution of GERD symptoms.[2]

Case 2. A 4-month-old girl, born preterm, was referred for evaluation of apnea and bradycardia spells present since birth. The infant averaged three spells per day and did not improve with caffeine therapy or following closure of a patent ductus arteriosus. Most spells followed feedings and usually resolved with cutaneous stimulation, although several sustained spells required cardiopulmonary resuscitation. Head ultrasound, magnetic resonance imaging, and electroencephalography failed to disclose the cause of the spells. Nasogastric feedings were initiated for poor weight gain, but did not alter the frequency of the apnea and bradycardia spells.

Barium esophagography with video swallow demonstrated primary aspiration on swallowing, but failed to detect reflux events. Extended pH probe monitoring detected GERD, and conservative and pharmacologic therapy (ranitidine and cisapride) were initiated. The number of apnea and bradycardia spells did not diminish, and a near-fatal apneic event prompted a direct laryngoscopy and bronchoscopy (DL & B) with esophagoscopy and biopsy. The laryngeal anatomy was normal, but histologic esophagitis confirmed the continued presence of GERD and the failure of medical antireflux therapy.

The apnea and bradycardia spells were again attributed to GERD, and a nasoduodenal feeding tube was placed. Remarkably, the spells ceased the following day. A fundoplication with a feeding jejunostomy tube was performed, and the infant was discharged to home 1 week postoperatively. Within 1 month, she experienced significant weight gain, ceased aspirating, and resumed oral feedings.

This case demonstrates that GERD may induce life-threatening respiratory events and that surgical antireflux therapy may successfully treat symptoms when medical therapy fails. Although the respiratory events may have arisen from primary (swallow) aspiration, it is more probable that they were reflex responses induced by stimulation of the distal esophagus by noxious refluxate. This is particularly likely as nasogastric feedings and cessation of oral feedings did not elicit improvement. Nasoduodenal feedings, by contrast, were highly effective and prompted complete resolution of the life-threatening symptoms. This suggests that delayed gastric emptying was a key predisposing factor in the infant's GERD. The nasojejunal feedings well predicted the resolution of symptoms that surgical antireflux therapy provided.

Case 3. A 13-month-old boy presented for evaluation of recurrent pneumonia and chronic nocturnal, nonproductive cough. The child required monthly hospitalizations for pneumonia and bronchospasm that began at several months of age. Pulmonary

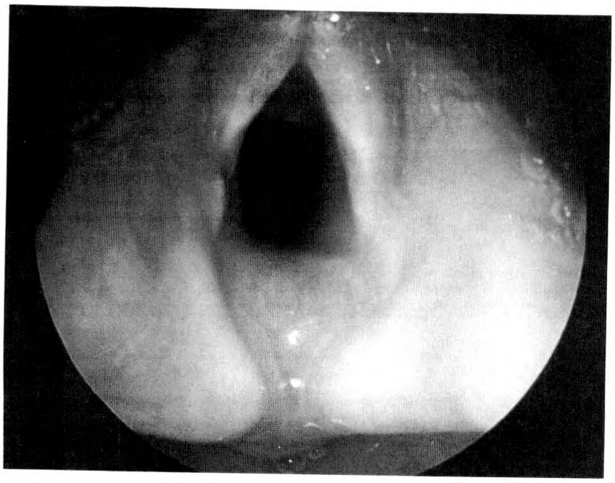

Fig 1. (Case 3) Type I laryngotracheoesophageal cleft in 13-month-old infant with gastroesophageal reflux disease (GERD), recurrent aspiration pneumonia, and chronic cough. Note absence of interarytenoid tissue above level of vocal folds. Endoscopic repair effected resolution of symptoms, which were attributed to aspiration of gastroesophageal refluxate.

function, immune response, sweat chloride, and cilia motility study findings were normal. Antibiotics, bronchodilators, and steroid therapy for presumed indeterminate asthma were of incomplete and short-lived benefit. Parental concern for the child's condition escalated, as his only sibling had died of sudden infant death syndrome (SIDS).

Aspiration was initially the suspected cause of recurrent pneumonia, but a barium esophagogram with video swallow failed to demonstrate the ingress of foreign material. The patient frequently awakened in a pool of emesis, and a diagnostic evaluation for GERD was initiated. An esophageal pH probe study demonstrated a borderline-positive Euler-Byrne score of 47. Conservative and pharmacologic (metoclopramide) antireflux therapy was initiated, but the chronic cough persisted and the child was readmitted for pneumonia. Repeat barium esophagogram with video swallow again showed no aspiration, but this time demonstrated significant GERD. Ranitidine was added to the GERD management, but without beneficial response. A modified Bernstein test failed to induce cough or bronchospasm, but the validity of the results was questioned as antireflux therapy had already been initiated.

We performed DL & B with bronchoalveolar lavage, as clinical suspicion of aspiration persisted. A type I laryngotracheoesophageal cleft was diagnosed (Fig 1) and aspiration was confirmed by a markedly elevated LLM score. The child's symptoms were thus attributed to a combination of GERD, as diagnosed by the barium esophagogram, and aspiration, as diagnosed by the elevated LLM index. Medical therapy for GERD was maximized with omeprazole and cisapride and was confirmed effective by subsequent normal findings on a pH probe study. Cough and recurrent pneumonia nevertheless persisted, and endoscopic repair of the type I laryngotracheoesophageal cleft was performed.

The child was extubated on the first postoperative day and discharged to home on the third. Antibiotics and steroid therapy were stopped but ranitidine was continued. Immediately postoperatively, the daily coughing paroxysms resolved. The child did remarkably well for the first 4 postoperative months. During the fifth postoperative month, he experienced return of his chronic cough, but without pneumonia. We confirmed by DL & B that the laryngotracheal cleft repair was intact. Repeat extended pH probe testing demonstrated excessive GER with a Euler-Byrne score of 263, but the child experienced minimal improvement on omeprazole. The child's recurrent cough was attributed to chronic sinusitis, which was not present during his initial evaluation. His symptoms resolved with therapy and he has returned to a state of good health.

This case demonstrates the difficulty in confirming the diagnosis of aspiration in pediatric patients. The LLM index was a helpful diagnostic tool, but did not differentiate primary (swallow) aspiration from secondary (GER) aspiration. It is more likely that secondary aspiration accounted for the patient's symptoms, as primary aspiration was never detected on multiple video swallow studies. The nocturnal nature of the child's cough also suggests secondary aspiration, as it coincides with the peak incidence of pediatric GER events. The type I laryngeal cleft likely rendered the larynx less competent and permitted aspiration, particularly during the night, when laryngeal neuromuscular tone diminishes and when reflux events increase.

The return of the chronic cough during the fifth postoperative month was attributed to chronic sinusitis, a diagnosis considered but excluded during the child's initial protracted preoperative evaluation.

Case 4. A 4-week-old infant presented for evaluation of upper airway obstruction due to Pierre Robin sequence. The infant required a tracheotomy for relief of upper airway obstruction and postoperatively continued to experience coughing paroxysms and oxygen desaturations despite the absence of pulmonary disease.

Barium esophagography confirmed GERD and a modified Bernstein test was performed to investigate a possible cause-and-effect relationship between esophageal acidification and the respiratory symptoms. Infusion of 0.1N hydrochloric acid (HCl) into the distal esophagus elicited irritability, a 9% decrease in baseline oxygen saturation, and a cough response that during the second HCl infusion was followed by emesis. Saline infusion failed to elicit cough, emesis, or oxygen desaturation, but did reduce the irritability initiated by the HCl infusion.

The modified Bernstein test was interpreted as positive, and the infant was treated with conservative and pharmacologic therapy (metoclopramide and ranitidine). The cough and oxygen desaturations resolved. Feeding difficulties persisted secondary to glossoptosis and necessitated a gastrostomy tube. The procedure was performed percutaneously without fundoplication, as the presumed GERD-induced respiratory symptoms resolved with medical management.

This case demonstrates the utility of the modified Bernstein test. Cough and oxygen desaturation were demonstrated to be temporally related to distal esophageal acidification. Saline infusion failed to evoke the respiratory symptoms and actually reduced the irritability of the infant following the HCl infusion. Medical treatment of GERD was effective and surgical antireflux therapy avoided.

DISCUSSION

Gastroesophageal reflux is a common phenomenon in neonates and young infants and diminishes as improved neuromuscular control allows infants to assume an upright posture. In some infants, GER persists or is severe enough to induce GERD and often must be treated. Historical data collected over 50 years ago attest to the morbidity of severe untreated GERD in infants and children with GI symptoms: GERD resolved by 2 years of age in 60% of patients, but persisted until 4 years of age in 30%. Half the remaining patients developed esophageal strictures and half died of inanition.[3]

There are numerous barriers against GERD, including the lower esophageal sphincter, the intra-abdominal esophagus, the angle of insertion of the esophagus into the stomach, distal esophageal motility, gastric emptying, and laryngeal competence. Inherent properties of the esophagus, such as intrinsic mucosal renewal, mucosal blood flow, and mucus secretion, also afford protection from noxious injury.

The lower esophageal sphincter is a zone of increased pressure that separates the positive pressure of the intra-abdominal cavity from the negative pressure of the thoracic cavity, thereby preventing reflux of gastric contents into the distal esophagus. Additional protection is provided by the intra-abdominal esophagus and the acute angle at which it enters the stomach, creating a sphincterlike flap that tightens when intra-abdominal pressure is increased. The lower esophageal sphincter is present in term and preterm neonates, although its intra-abdominal portion is poorly developed at birth and of reduced efficiency until approximately 6 weeks of age.[4] The lower esophageal sphincter is under hormonal and neural control, and its tone reflexively increases with elevations in gastric pressure.[5] Decreases in lower esophageal sphincter pressure are induced by many medications and foods, including theophylline, beta agonists, calcium channel blockers, narcotics, benzodiazepines, anticholinergics, chocolate, fats, caffeine, licorice, carbonated beverages, alcohol, and nicotine.[6]

Distal esophageal motility serves as a protective mechanism against GERD by clearing the esophagus of refluxed gastric contents. Unlike reflux events in adults, most episodes in pediatric patients occur during sleep, when clearance of refluxate is reduced.[7] Clearance is dependent on the rate of swallowing and the efficacy of esophageal peristalsis, both of which significantly decrease during sleep in infants and children with GERD compared to asymptomatic controls.

Gastric motility also reduces the incidence of reflux events, and delayed gastric emptying may predispose to GERD.

Although normal esophageal anatomy and physiology reduce the frequency of reflux events and expedite their clearance, it is the esophageal mucosal barrier that likely prevents reflux episodes from inciting local pathologic changes. If the esophageal mucosal barrier is violated, inflammation results, peristalsis diminishes, and potent reflexes may be elicited.

Respiratory symptoms induced by GERD include recurrent pneumonia, bronchitis, asthma, central or obstructive apnea, stridor, and chronic cough. As a cause-and-effect relationship becomes better defined, more children with GERD are evaluated for respiratory problems. The percentage of children with GERD-induced respiratory problems has reached approximately 30%, three times the estimate 15 years ago.[8,9] Emesis or visible signs of reflux often are absent in these children, adding to the difficulty of diagnosis.

Recurrent aspiration-induced pneumonia and bronchitis suggest possible laryngeal incompetence (case 3). Chronic lung disease from repeated aspiration of refluxate may develop, with radiographic findings consistent with bronchopulmonary dysplasia.[10] Declining pulmonary status or the presence of gastric contents in tracheal aspirates of infants with bronchopulmonary dysplasia should raise the suspicion of GERD. Antireflux treatment may result in marked improvement in pulmonary function and radiographic findings.

Reflex bronchospasm due to GERD should be suspected in children with asthma that does not respond to medical therapy, especially if the history is negative for atopy and symptoms are primarily nocturnal. Sir William Osler[11] was one of the first physicians to recognize this relationship. He noted that asthmatics consumed their largest meal at noon to avoid the nocturnal wheezing that followed a more plentiful evening meal. Microaspiration was believed to be the underlying pathologic event; however, esophageal acidification in the absence of aspiration also has been demonstrated to cause bronchoconstriction.[12] Inflammation and possible disruption of the esophageal mucosal barrier may predispose to sensory nerve stimulation and subsequent reflex bronchoconstriction.[6]

Bronchoconstriction induced by GERD is more likely to occur at night for several reasons. Reflux events increase nocturnally, and esophageal acid clearance is delayed. Additionally, the efficacy of bedtime bronchodilators wanes, and serum epinephrine reaches its nadir at 4:00 AM. The important interplay of these factors has been demonstrated clinically by the modified Bernstein test in asthmatic children with confirmed GERD.[13]

In some infants and children, GERD may cause life-threatening central or obstructive apnea with associated cardiovascular responses.[14] Obstructive apnea following a drop in distal esophageal pH can be demonstrated in infants undergoing simultaneous polysomnography and esophageal pH monitoring. Central apnea and bradycardia spells also can be seen (case 2).

These responses may be reflex-mediated, as demonstrated in the esophagolaryngeal adductor reflex and the laryngeal chemoreflex.[15,16] Both reflexes have been extensively studied in animals, and clinical data in neonates and infants suggest that similar reflexes occur in humans. The esophagolaryngeal adductor reflex is induced by distal esophageal sensory nerve stimulation and results in intense laryngeal adduction or laryngospasm, tachycardia, and hypertension[15] (also Sandler et al, unpublished observations). The laryngeal chemoreflex is induced by direct laryngeal stimulation with water or dilute acid.[16] Like the esophagolaryngeal adductor reflex, the laryngeal chemoreflex peaks in immature animals, but unlike the esophagolaryngeal adductor reflex, it is characterized by central apnea, bradycardia, and hypotension with rebound hypertension. The esophagolaryngeal adductor reflex shares noteworthy features with apparent life-threatening events and SIDS. Both the esophagolaryngeal adductor reflex and SIDS are age-dependent events that peak in immature animals. Laryngospasm may be a component of SIDS, as postmortem pulmonary and thymic petechiae are often seen, suggesting preterminal vigorous respiratory efforts against a closed glottis.[17]

The role of GERD in causing or exacerbating stridor presents a diagnostic challenge. In infants with laryngomalacia, GERD may transform "noisy breathing" into hypoxic events.[18] Stridor also may occur following reflux events in the absence of laryngeal anomalies.[19]

Chronic cough may be caused by occult GERD, particularly in children less than 18 months of age.[20] Typically, children will have received an extensive pulmonary evaluation prior to referral to an otolaryngologist, and a high index of suspicion is necessary to achieve the diagnosis. Cough and GERD may result in a vicious cycle whereby coughing elevates intra-abdominal pressure and encourages reflux events that in turn predispose to more coughing (case 1).

The role of GERD in causing subglottic stenosis or tracheal granulation tissue after intubation or laryngeal trauma has been explored in animal models and in children who require laryngotracheal reconstruction.[21,22] To optimize surgical results, management of GERD has been demonstrated to be an important component of postoperative care. In these patients, GERD is particularly likely, as nasogastric tubes, narcotics, and neuromuscular blockade promote GER (Bauman et al, unpublished observations).

Specific anatomic abnormalities of the aerodigestive tract can predispose to GER and GERD. One of the better-described abnormalities is tracheoesophageal fistula with esophageal atresia. Distal esophageal motility is poor and lower esophageal sphincter tone is diminished, making GERD a common occurrence.[23] The result can lead to stricture formation following tracheoesophageal fistula repair or respiratory complications of GERD. The latter warrants the exclusion of a laryngotracheoesophageal cleft, a rare anomaly often seen in association with tracheoesophageal fistulae.[24]

Lesser degrees of laryngeal clefting (type I) have been described and also can lead to GERD-induced respiratory problems. Minor clefts are probably more common than previously recognized, and instruments to facilitate their endoscopic diagnosis are being developed.[25] Case 3 illustrates the importance of recognizing type I laryngotracheoesophageal clefts,

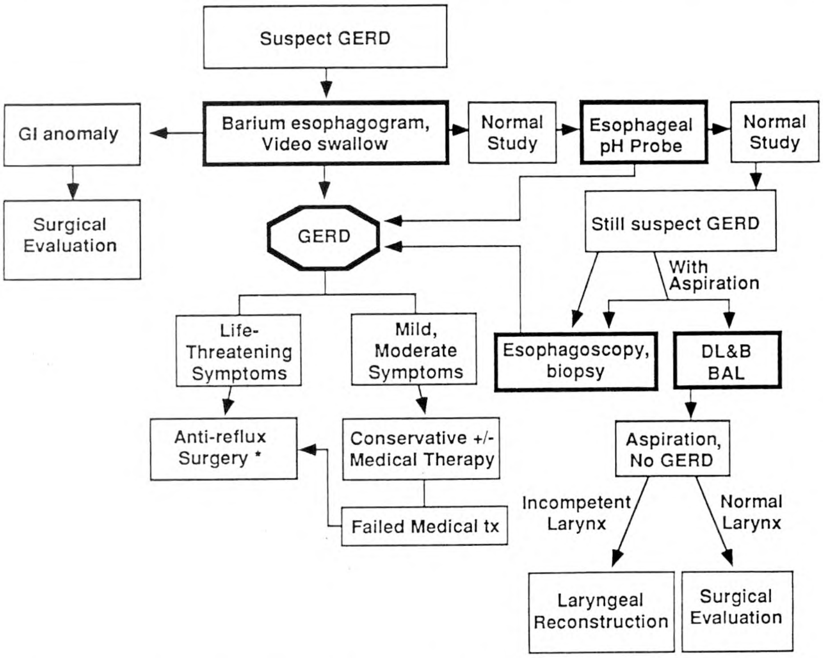

Fig 2. Approach to diagnosis and management of pediatric patients with symptoms of GERD. Diagnostic procedures are indicated in bold rectangles. DL & B — direct laryngoscopy and bronchoscopy, BAL — bronchoalveolar lavage, tx — therapy, Anti-reflux surgery* — neurologically compromised children may benefit from feeding jejunostomy tube.

particularly in the presence of GERD-induced respiratory problems.

Our approach to the diagnosis of GERD is illustrated in Fig 2. Infants and children presenting with mild, uncomplicated GI symptoms, such as regurgitation, may be treated conservatively without prior diagnostic evaluation. All other infants and children with suspected GERD first are evaluated by combined barium esophagography with video swallow. Anatomic abnormalities that may mimic or cause GI manifestations of GERD, as well as aspiration during the oral phase of swallowing, may be detected. If reflux is present in the absence of anatomic abnormalities, conservative, pharmacologic, or surgical therapy is initiated and guided by the severity of the symptoms and by the child's health status.

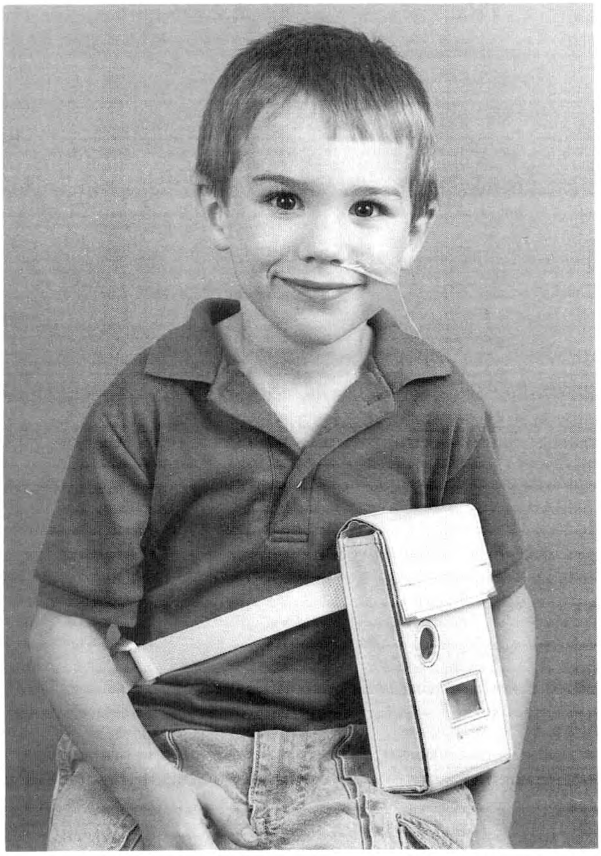

Fig 3. Six-year-old child undergoing extended distal esophageal pH monitoring with flexible probe passed through left naris.

Extended distal esophageal pH monitoring is performed if barium esophagography does not identify suspected pathologic reflux. Distal pH monitoring is a more sensitive study than barium esophagography and typically is performed by the pediatric GI service. It may also provide a potentially useful reference to assess the efficacy of surgical therapy for GERD. The procedure is usually performed on an inpatient basis, as recordings are obtained over a 16- to 24-hour period. The tip of the pH probe is calibrated and passed through the naris under fluoroscopic control to confirm its position at 87% of the distance from the external naris to the lower esophageal sphincter (Fig 3). The narrow probe is well tolerated above the lower esophageal sphincter and does not interfere with sphincter competency. A nurse or caregiver maintains a diary of activities, recording the child's position, meals, activity, and appearance of symptoms every 15 minutes. Episodes of pH ≤ 4.0 are registered as reflux events and the frequency and duration are recorded. This value correlates with the onset of pyrosis in adults and with the conversion of

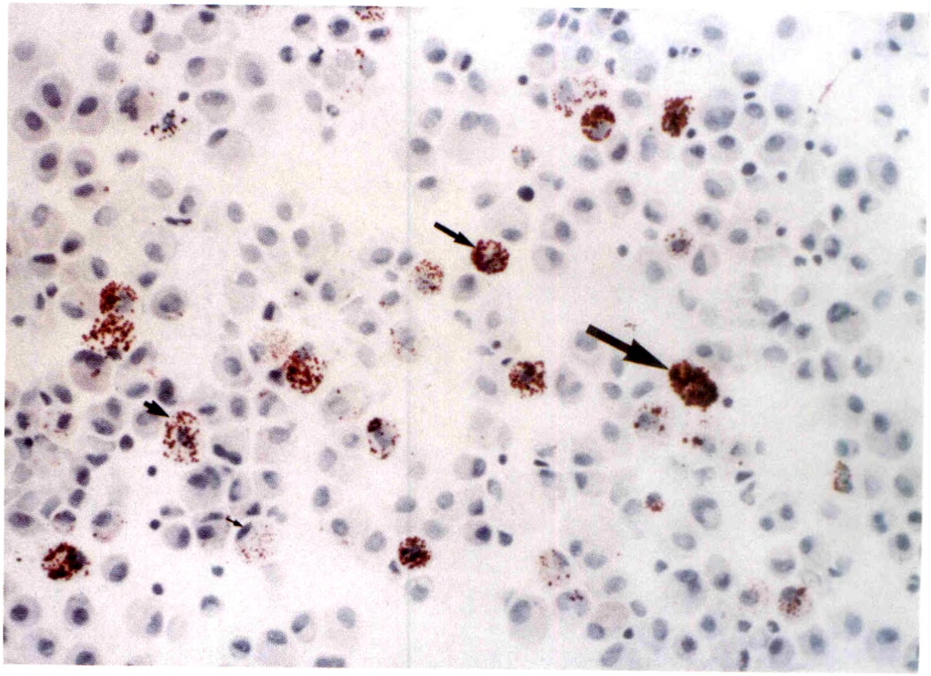

Fig 4. Intracellular lipid demonstrated in alveolar macrophages stained with oil red O and scored from 1 (smallest arrow) to 4 (largest arrow). One hundred macrophages are assigned score from 0 (none) to 4 and tallied. Total scores greater than 70 are indicative of aspiration.

pepsinogen to pepsin.[26]

A multivariate analysis comparing the episodes of GER in symptomatic and asymptomatic infants established the Euler-Byrne formula to differentiate the two groups.[27] The Euler-Byrne score is defined as x + 4y, where x equals the number of episodes that distal esophageal pH is less than 4 and y equals the number of episodes in which the pH remains less than 4 for greater than 5 minutes. A score greater than 50 is considered diagnostic of pathologic reflux.

Suspected aspiration may be confirmed by bronchoalveolar lavage for LLM. At bronchoscopy, free alveolar macrophages are collected and assessed for intracellular lipid, a sign of aspiration. The procedure is performed by instilling 5 to 7 mL of saline into the subsegmental bronchi and collecting it by gentle suctioning, repeating until approximately 5 mL of aspirate have been retrieved. After centrifugation and staining with oil red O to identify lipid, 100 macrophages are counted and assigned a score from 0 (lowest) to 4 to reflect the amount of intracellular lipid (Fig 4). A total LLM score greater than 70 is considered evidence of aspiration.[28] The minimal amount of refluxate that must be aspirated to increase the LLM to >70 has not been established, but it is estimated that the LLM index becomes positive 6 hours after a reflux event and will remain positive for 3 subsequent days.[29] Studies have demonstrated a strong correlation with lactose assay following milk aspiration; however, aspirated gastric contents cannot be differentiated from aspirated oral feedings.[30] In spite of this limitation, bronchoalveolar lavage for LLM is performed easily and is a useful test in the evaluation of GERD-induced respiratory disease. Concurrent esophageal biopsies also are useful, especially in the rare instances in which pH probes are falsely negative.

Concomitant DL & B may reveal laryngeal anomalies that render the glottis incompetent and prone to aspiration. Most important, a type I laryngotracheoesophageal cleft must be excluded, preferably by measuring interarytenoid notch height. Preliminary studies have shown that in the presence of GER, a low interarytenoid notch height predisposes an infant or child to GERD-induced respiratory disease.[25] Even if findings of pH probe monitoring are normal, DL & B, with bronchoalveolar lavage, are useful procedures, as aspiration of infrequent reflux events can produce respiratory or laryngeal symptoms. Typically, posterior laryngeal structures are most vulnerable to irritation by GER. The posterior glottis can be edematous or erythematous, interarytenoid tissue can be hypertrophic, and the vocal folds can develop granulomas.

Many studies are available to detect pathologic GER, but the modified Bernstein test is the only study that confirms a cause-and-effect relationship between esophageal acidification and symptom manifestation. The Bernstein test was originally developed in adult patients to differentiate cardiac from reflux-related chest pain, but has been modified for pediatric patients, in whom chest pain is an unusual manifestation of GERD. Ten-minute infusions of 0.1N HCl (0.05 mL/cm of height per minute) through a nasogastric tube into the distal esophagus of a monitored patient are alternated with saline for a total of four infusions.[19] The appearance of the symptom in question following acid infusion and its absence following saline infusion denotes a positive modified Bernstein test and establishes GER as the cause of the symptom. The modified Bernstein test may be particularly helpful in determining the cause of stridor, central or obstructive apnea, or oxygen desaturation in pediatric patients.[19,31]

In most infants, GERD resolves within the first 2 years of life. However, interim antireflux treatment may be required in some infants to treat GERD and prevent sequelae. Mild symptoms are treated by conservative or nonpharmacologic therapy, more significant symptoms are treated with antireflux or acid-reducing medications, and life-threatening or pharmacologic-resistant symptoms are treated surgically.

Conservative therapy entails proper positioning and dietary modifications. Postprandial prone positioning in infants with GERD markedly reduces reflux events, improves gastric emptying, decreases aspiration, and reduces energy expenditure.[6,32] Head of bed elevation, as once encouraged, may not provide more benefit than prone positioning for infants.[33] Upright postprandial positioning in a car seat should be discouraged, as infants assume a slumped position that submerges the gastroesophageal junction into the stomach and promotes reflux events.[32] Older children, however, have adequate neuromuscular control and should remain upright during the postprandial period to decrease reflux events.

Dietary modifications recommended to diminish GERD include formula thickening, small frequent feedings, and avoidance of foods that decrease lower esophageal sphincter pressure. Formula thickening with rice cereal increases the viscosity of gastric contents and should theoretically diminish reflux events. Although emesis or visible reflux is decreased with thickened formula, pH probe monitoring has demonstrated that the number of nonregurgitant reflux events actually increases, particularly during the first 2 postcibal hours.[6] Nonetheless, thickened feeds often are recommended because of their confirmed advantages of improved caloric content, increased sleeping time, and decreased crying time.

The benefit of small, more frequent feedings has not been adequately established. The number of postprandial reflux events per feeding may indeed decrease with smaller feeding volumes, but the number of postprandial periods during which reflux can occur increases. Small frequent feedings currently are recommended for infants with GERD who consume excessively large feedings.[34] Without controversy, older children with GERD should reduce their nocturnal gastric volume by fasting for several hours prior to bedtime.

A 7- to 10-day course of nasogastric tube feedings may also effectively treat GER-induced failure to thrive[2] (case 1). Sustained benefit from this therapy is expected in infants who demonstrate improved growth after 1 week of feedings (case 3). Infants who do not benefit from nasogastric feedings may still experience symptom resolution with nasoduodenal feedings (case 2). Such infants likely have delayed gastric emptying that predisposes them to GERD.

If symptoms persist or worsen despite conservative measures and diagnostic studies confirm GERD, pharmacologic therapy should be initiated. Pharmacologic therapy focuses on improving GI motility, augmenting lower esophageal sphincter pressure, and/or reducing gastric acidity. Prokinetic agents usually are administered first, and if they are poorly tolerated or if symptoms of reflux disease persist, acid-reducing medications are added.

Metoclopramide (0.1 mg/kg four times per day; before meals and at bedtime) is a commonly used prokinetic agent in pediatric patients. It increases lower esophageal sphincter pressure, esophageal contractions, gastric fundic pressure, and gut coordination.[35] Cholinergic effects are largely restricted to the proximal gut and require an intact cholinergic system. The most common side effects are in the central nervous system and include nervousness, somnolence, and dystonic reactions. Alarming extrapyramidal signs usually begin 24 to 48 hours after initiating therapy and include involuntary limb movements, facial grimacing, torticollis, and oculogyric crisis. These side effects are more common in pediatric patients and usually respond quickly to drug withdrawal and diazepam or benztropine mesylate. Metoclopramide should not be administered with phenothiazines or thioxanthenes.

Cisapride (0.2 mg/kg four times per day; before meals and at bedtime) is a newer prokinetic agent with several attractive features compared to metoclopramide.[36] It is a noncholinergic agent that enhances postganglionic release of acetylcholine. Like metoclopramide, it augments lower esophageal sphincter pressure and improves GI motility and coordination. Unlike metoclopramide, it lacks central dopamine antagonism and has neither antiemetic properties nor extrapyramidal side effects. Cisapride is a valuable first-line agent in the treatment of GERD. Severe side effects have not been reported, and mild side effects, such as headache, drowsiness, colic, and diarrhea, are rare.[36]

Should symptoms of GERD persist despite the use of a prokinetic agent, or if signs of esophagitis are present, therapy is aimed at reducing the acidity of the refluxate. An H2 receptor antagonist should also be administered with the prokinetic agent. Ranitidine, cimetidine, and famotidine are H2 antagonists that decrease parietal cell gastric acid secretion by approximately 70%. Although the frequency of GER is not decreased, reduced acidity lessens the symptoms of GERD.[37] Ranitidine (2 mg/kg twice per day, orally) and cimetidine (5 to 10 mg/kg every 6 hours, orally) are the most commonly prescribed H2 receptor antagonists.

If control of GERD is not achieved with the prokinetic agent and H2 receptor antagonists, omeprazole therapy may be considered. Omeprazole acts as a hydrogen ion pump inhibitor by blocking sodium- and potassium-activated adenosine triphosphatase and is the most complete inhibitor of gastric acid secretion. It is effective in treating severe esophagitis in pediatric patients unresponsive to H2 receptor antagonists.[38] Additional studies probably will demonstrate its success in the treatment of atypical symptoms of GERD. Omeprazole is recommended only for short-term usage because of potential gastrinoma formation from induced hypergastrinemia. Pediatric dosing data are not yet available.

Antacids (0.5 mL/kg after meals and at bedtime) and mucosal protective agents like sucralfate are effective in the treatment of esophagitis, but their role in reducing GERD-induced respiratory symptoms remains to be determined. Antacids require frequent administration after each meal and before bedtime, which may reduce compliance.

Surgical therapy is advised if pharmacologic therapy fails to improve GERD or if manifestations of GERD are life-threatening. Fundoplication is a relatively common procedure in large patient centers. Potential side effects, such as gas-bloat syndrome, inability to belch or vomit, and acute gastric distention, are markedly reduced by a partial wrap of the gastroesophageal junction. Neurologically impaired children with dysmotility may benefit less from a complete fundoplication and should undergo a partial fundoplication with gastrostomy or, alternatively, a gastrostomy with a feeding jejunostomy.

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LONG-TERM EFFECTS OF BOTULINUM TOXIN INJECTIONS IN SPASMODIC DYSPHONIA

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The purpose was to examine whether physiological changes can be found in laryngeal muscles following repeated treatment with botulinum toxin injections in spasmodic dysphonia. Seven patients whose treatment consisted of multiple unilateral thyroarytenoid injections were examined more than 6 months following their most recent botulinum toxin injection by fiberoptic laryngoscopy and electromyography. Comparisons were made between injected and contralateral noninjected muscles' motor unit characteristics, muscle activation patterns, and vocal fold movement characteristics. The results demonstrated that motor unit characteristics differed between injected and noninjected muscles and that these differences were greater in patients less than 12 months since last injection. Motor unit duration differences were reduced and motor unit amplitude and numbers of turns were increased in muscles sampled over 1 year after injection. These results suggest that while the physiologic effects of botulinum toxin are reversible, the reinnervation process continues past 12 months following injection.

KEY WORDS — botulinum toxin, spasmodic dysphonia.

INTRODUCTION

Spasmodic dysphonias (SDs) are idiopathic vocal fold movement control disorders acquired in adulthood usually following an upper respiratory tract infection or during a period of emotional stress,[1] or infrequently following head trauma.[2] The SDs are thought to be focal dystonias similar to blepharospasm, oromandibular dystonia, torticollis, and writer's cramp, dystonias often associated with SD.[3] The most common form of SD is adductor SD, characterized by difficulty with voice initiation,[4] a strain-strangle voice pattern, phonatory and pitch breaks, and glottal fry.[1] No laryngeal abnormalities are noted at rest during fiberoptic nasolaryngoscopy of patients with SD. However, during speech, intermittent hyperadductions of the glottal and ventricular folds become evident.[5] Studies examining motor unit characteristics during laryngeal electromyography (EMG) have noted abnormalities including enlarged potentials, polyphasics, reduced numbers of motor units, and small potentials in some SD patients.[6] Studies examining muscle activation patterns have not found consistent differences between SD and the normal state. Some report hyperactivity in the thyroarytenoid (TA) muscles,[7,8] while others have been unable to find quantitative differences from normal muscles, either in the pattern or amplitudes of laryngeal muscle recordings during speech.[9,10]

Over the last 10 years, localized injections of botulinum toxin have become the treatment of choice for controlling symptoms in patients with focal dystonias.[11-18] In treating SD, the TA muscle on one or both sides is injected.[19,20] Short-term complications are usually mild and consist of transient dysphagia and breathy phonation.[13,19] No long-term complications have been reported.[11] Symptoms usually return at 4 to 6 months, and reinjection is required.[19]

Botulinum toxin produces denervation by irreversible binding to the presynaptic terminals, thus decreasing the amount of acetylcholine released in response to a nerve action potential.[21] Within 2 weeks, the muscle fibers begin to atrophy, and over this same time period, symptoms and signs of hyperadduction are reduced. Following botulinum toxin injection, two patterns of axonal outgrowth are stimulated: 1) collateral sprouting, which increases the size of the end plate region, and 2) noncollateral sprouting from the tips of motor axons and nodes of Ranvier, which produces reinnervation.[22] Although no muscle fiber architecture alterations have been reported after use of botulinum toxin,[23-25] studies of the effects of repeated injections of the orbicularis occuli muscles for blepharospasm have demonstrated irreversible neurohistologic abnormalities.[26] Axonal sprouts can reestablish themselves in proximity to neuromuscular junctions, or end blindly, and these abnormalities have been described up to 3 years after botulinum toxin injection.[27] Other changes have included the presence of thin unmyelinated axonal collaterals contacting end plates, an increased number of muscle fibers innervated by individual terminal motor axons, a profusion of unmyelinated axonal sprouts, a variability in end plate sizes, and multiple end plates on muscle fibers.[26] These histologic changes could re-

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TABLE 1. PATIENT CHARACTERISTICS

Patient No.	*Age (y)*	*Thyroarytenoid Muscle Injected*	*No. of Treatments*	*Total Dose (LD50 Mouse Units)*	*Time Since Last Injected (mo)*	*Glottic Motion*
1	39	L	7	100	13	Asymmetric
2	34	L	7	142.5	6	Asymmetric
3	53	L	2	100	38	Normal
4	36	R	3	40	7	Normal
5	74	L	5	65	9	Asymmetric
6	44	L	3	42.5	29	Normal
7	50	L	5	67.5	7	Asymmetric

sult in physiologic changes in motor unit characteristics.

In light of these reports describing histologic changes in muscles after botulinum use for blepharospasm, we undertook a study to determine whether repeated use of botulinum in the treatment of adductor SD results in persistent physiologic abnormalities. This was evaluated by laryngeal EMG studies of injected and noninjected laryngeal muscles. Because of previously noted motor unit abnormalities in untreated patients with SD, physiologic characteristics of injected muscles could not be compared with those of normal controls. Rather, we chose to compare previously injected TA muscles with their contralateral noninjected TA in patients undergoing repeated unilateral botulinum injections. Patients were studied at least 6 months following botulinum toxin injection so that adequate time for reinnervation should have passed and any possible systemic effects could have reversed.[21] Although botulinum toxin has been observed to spread through muscle fascia[28] and from the TA muscle to the lateral cricoarytenoid muscle on the same side in the larynx,[29] spread to the TA muscle on the opposite side of the larynx is unlikely. Parameters analyzed included individual motor unit characteristics and total EMG activity. A degradation in EMG activity or a change in motor unit characteristics on the side of previous injection may be physiologic evidence of laryngeal muscle abnormalities resulting from repeated botulinum toxin use.

METHODS

Seven patients with adductor SD were studied (Table 1) after signing an informed consent for participation in a protocol approved by a National Institutes of Health Internal Review Committee. Each had previously received between 2 and 7 unilateral TA muscle botulinum toxin injections. The average treatment dosage was 15 LD50 mouse units and ranged from 10 to 50 units, resulting in total dosages across all treatments from 40 to 142.5 units over 21 to 47 months. The EMG testing was done between 6 and 38 months following the last botulinum toxin injection.

Flexible fiberoptic videotapes were viewed from pretreatment recordings and recordings done at the time of EMG testing by two observers. The vocal fold position at rest and during phonation and inspiration was reviewed, and the motion described as either normal (symmetric) or asymmetric.

Prior to each EMG recording session, 2-V peak to peak calibration signals were recorded. Following an intradermal injection of 2% lidocaine into the skin overlying the cricothyroid space, electrodes were inserted into both TA muscles by means of standard techniques[30] to approach the TA muscle and avoid the lateral cricoarytenoid. Concentric needle electrodes (DISA 13L50), 27-gauge with a 0.07-mm^2 recording surface, were used in all patients except patient 4, who was tested with 27-gauge bipolar needle electrodes (DISA 13K80) that had a 0.015 mm^2 recording surface. Accurate placement of electrodes into the TA muscle was assured by verifying gestures, including effort closure and sustained vowel phonation. With correct placement, both gestures caused increased muscle activity that remained active during vocal fold closure. When activity was not maintained throughout phonation or effort closure, placement in the lateral cricoarytenoid muscle was indicated, and the placement was changed to a more superior and medial location.

The following procedures were carried out to provide recordings from three different locations in each TA muscle, injected and noninjected, during an EMG recording session. After verification of the correct placement of the two electrodes, EMG signals were band-pass filtered between 100 and 5,000 Hz, amplified, and recorded on FM tape along with speech acoustics and respiratory movements. The first recording was made from the posterior aspect of a TA muscle. The EMG recordings from this site included 1 minute of quiet respiration followed by three or more samples of prolonged phonation of the vowel /i/ at a high chest-register pitch and moderate intensity. The electrodes were then withdrawn to another region in the middle portion of each TA muscle, and quiet respiration and phonation were again recorded.

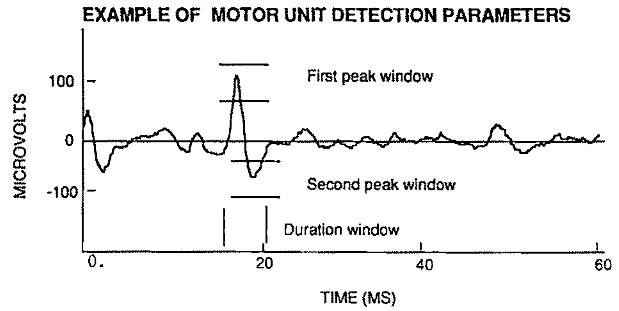

Fig 1. Illustration of detection parameters used to identify recurrences of individual motor unit: directional requirement (positive- or negative-going), amplitude bounds window for acceptable peak amplitudes, and duration bounds window for acceptable durations between time of first and second peaks.

Anterior sites were similarly recorded. In some patients, a second anterior site still within the TA muscle could be recorded. Following the removal of the electrodes, testing was complete.

The EMG signals were digitized at 10,000 samples per second (sps) with anti-aliasing (low pass) filtering at 4,000 Hz. Speech was digitized at 10,000 sps with filtering at 5,000 Hz. Respiratory movement was filtered at 200 Hz and digitized at 500 sps. Review of the raw EMG signal by oscilloscope allowed the selection of specific segments of the signal for digitization. These included 20 seconds of quiet respiration without evidence of voluntary EMG activity. Phonation was digitized at areas of maximal EMG activity, near the onset of phonation. By means of the digitized calibration signals, the EMG signals were converted into microvolts by linear interpolation.

Individual motor units and total EMG activity were analyzed by signal stream processing software (Mitsyn) as previously reported.[31] Individual motor unit firings were first visually identified on the computer screen from the EMG recordings made during quiet respiration. After finding several identical motor unit firings within an original stream, the amplitudes and directions (positive or negative) of the first and second peaks and the time duration between the two peaks were measured for that unit. These parameters were then entered into the software program designed to select motor units by searching for the directions of the first and second peaks, the peak amplitude bounds of each, and the time bounds for the duration between the first and second peaks (Fig 1). Peak directions, amplitude windows, and time windows could be changed when using the program to find a different motor unit with different characteristics within the same or a different signal stream. Only units that could be identified in at least 10 locations in a 10-second resting EMG signal were used for analysis.

As firings of a unit were detected by the software, their peak-to-peak amplitudes and durations were measured. The durations were the time (in milliseconds [ms]) between the two largest adjacent peaks in a unit. Thus, while the vast majority were biphasic, the durations of biphasic, triphasic, and polyphasic units were measured similarly. Usually the two largest adjacent peaks were the first two peaks of a unit. Data obtained by these measures were later used to calculate the mean duration and amplitude for each motor unit identified. Standard deviations of unit durations were also calculated.

The EMG activity was assessed by software designed to quantify the numbers of turns in a signal. Turns refers to the number of direction changes in an EMG signal in which the amplitude between direction changes is greater than 50 μV (Fig 2). This measure reflects the numbers of large units being fired at a recording site during muscle activation.[32,33] Software was written to detect sequences of successive peak-to-peak differences greater than 50 μV. In quiet respiration signals, the EMG recording was previewed and the quietest areas were selected for analysis by the turn-counting program. For each signal, three separate 1-second periods were analyzed. For the phonation signals, areas of maximal activity near the onset of each phonation were selected. The number of turns at rest was subtracted from the number during phonation to remove the effect of baseline EMG activity seen during quiet

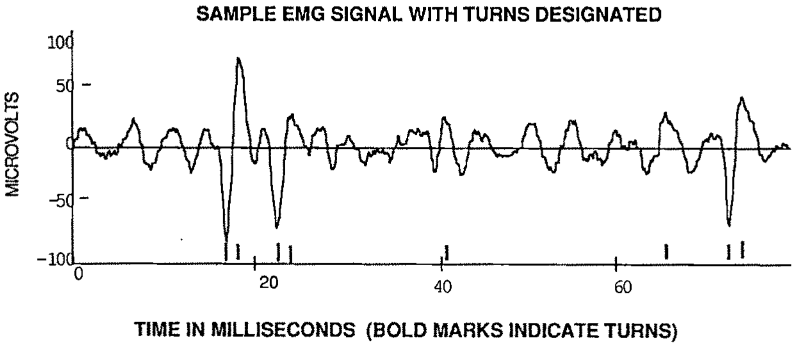

Fig 2. Illustration of detection method used to identify turns in electromyographic signals. Successive crossings had to differ by more than 50 μV for turn to be detected.

TABLE 2. MEAN MOTOR UNIT CHARACTERISTICS AND TURN ANALYSES BY PATIENT

Patient No.	*Side*	*No. of Motor Units*	*Duration* (ms)*	*Mean Amplitude (μV)*	*Mean No. of Turns per Second*
1	Injected	12	1.769 ± 0.837	389	612
	Noninjected	15	1.991 ± 0.642	209	192
2	Injected	4	4.697 ± 1.678	40	80
	Noninjected	8	2.344 ± 0.871	405	46
3	Injected	10	3.351 ± 1.174	122	232
	Noninjected	10	2.031 ± 0.863	172	326
4	Injected	8	1.757 ± 0.359	238	418
	Noninjected	21	1.612 ± 0.536	180	939
5	Injected	7	4.915 ± 1.535	196	272
	Noninjected	10	4.206 ± 1.726	160	385
6	Injected	7	3.375 ± 0.982	621	618
	Noninjected	4	3.579 ± 1.145	45	232
7	Injected	3	6.697 ± 2.588	103	188
	Noninjected	9	3.732 ± 0.915	140	305

*Mean ± SD.

respiration. The difference in turns for phonation was then compared between injected and noninjected muscles.

Before each statistical analysis, the distribution of each of the variables was examined. If this was not normal, then logarithmic transformations were used before the statistical comparisons were conducted. Because the motor units and samples for counting turns were made from various locations in muscles, these were assumed to be independent measures, while subject effects were included as a blocking factor in the MANOVAs.

RESULTS

On review of the pretreatment entry video recordings, no patient was found to have vocal fold motion asymmetry. Various degrees of hyperadductory spasmodic motion were noted; however, this was judged to be symmetric as previously described in adductor SD.[6] Following repeated injections of botulinum toxin, 4 of the 7 patients were found to have vocal fold motion asymmetry despite a 6- to 13-month hiatus since last injection (Table 1). In 3 of these patients, the injected vocal fold was shorter than the opposing fold at rest during quiet respiration. The other patient had asymmetry evident only during vocal fold motion. None of those with symmetric motion evidenced vocal fold shortening. Patients with vocal fold asymmetry tended to be those treated with a greater number of injections (Mann Whitney U = 12, p = .057). The total botulinum dose, however, did not appear to relate to vocal fold asymmetry.

We were able to identify 128 individual motor units from the EMG recordings made at rest. Of these, 51 were found in treated TA muscles and 77 in untreated muscles. The numbers of units identified in the injected and noninjected TA in each patient and their mean characteristics are listed in Table 2.

Almost all of the units found in this study (124 out of 128) had biphasic or triphasic patterns similar to those previously reported.[34,35] Only 4 units qualified as polyphasics, by having five or more phases identified.[36] One polyphasic unit was found in an injected muscle, and 3 were in noninjected muscles. Thus, for injected and noninjected muscles, the frequency of polyphasic units was similar to that reported for normal TA muscles.[34,35] Further, motor unit amplitudes (mean, 226 μV; range, 15.8 to 2,240 μV) fell within the normal range as described by previous authors.[34,35]

Table 2 presents the mean values for each of the patients. Because patient 4 was sampled with a bipolar electrode while all others were sampled with a concentric electrode, we tested for significant differences between this patient and the others in units from injected and noninjected muscles. Significant differences were found in the mean durations in noninjected (t = 3.863; p = .0005) and injected muscles (t = 2.73; p = .009) and in the standard deviations for durations in noninjected (t = 3.411; p = .001) and injected muscles (t = 3.97; p = .0005). No significant differences were found in the mean amplitude or the standard deviation in mean amplitude in either noninjected or injected muscles. The numbers of turns differed significantly between patient 4 and the other patients in the noninjected muscles (t = –10.991; p = .0001). No significant differences in electrode type were found in the injected muscles. However, when the mean values for each of the patients presented in Table 2 are examined, the values for patient 4 are very similar to

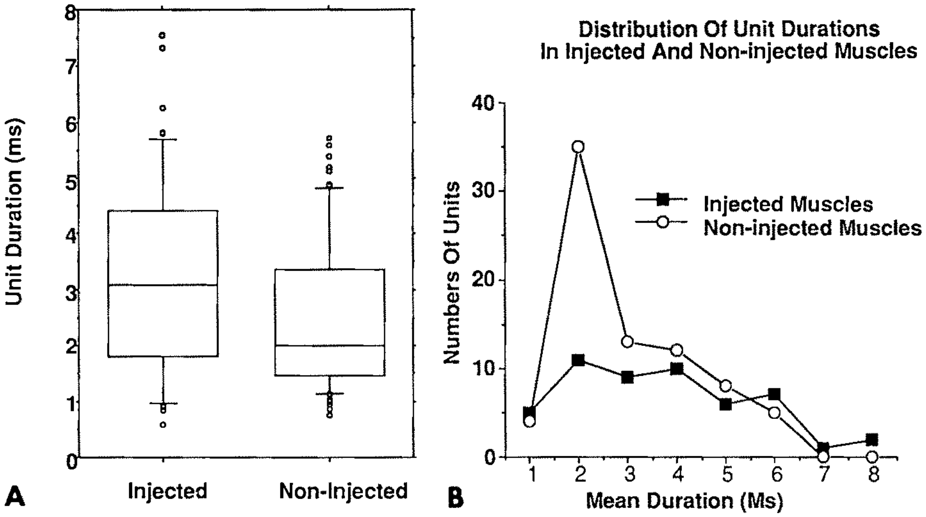

Fig 3. Motor unit durations in injected and noninjected muscles. A) Box plots showing median and quartile distributions for mean durations measured from motor units in injected and noninjected thyroarytenoid muscles. B) Frequency distributions of numbers of units with mean durations between 0 and 1, 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7, and 7 and 8 milliseconds in injected and noninjected muscles.

those for patient 1, while there are large interpatient differences. Therefore, in all analyses of group data, the effects of patient differences were tested to examine for differences between patients and how this affected the main effects of muscle injection.

Comparisons Between Injected and Noninjected Muscles. The mean durations of motor units found in injected and noninjected muscles were compared. Each unit was coded as to whether it was thought to be from a sample made in the anterior, middle, or posterior portion of the TA muscle. Because more were thought to be from the anterior position, the data were classified as coming from either the anterior portion or other portions (the middle and posterior regions combined). Then ANOVAs were computed to examine treatment effects (injected versus noninjected) while correcting for differences between patients and sampling sites. Because a different electrode was used in patient 4, any differences between this patient and others were examined when patient differences were included in the ANOVA model. The patient differences were significant ($F = 27.478$; $p \leq .0005$), as were sampling site effects within patients ($F = 2.567$; $p = .017$). After correcting for these differences, overall treatment effects were tested and found to be significant ($F = 28.615$; $p \leq .0005$). These were due to longer durations seen in the injected muscles (injected mean, 3.25 ms; noninjected mean, 2.5 ms; Fig 3A). The distribution of motor unit durations showed a loss of the short-duration units (<2 ms) in the injected muscles (Fig 3B). Comparison of these overall distributions by contingency analysis revealed a trend that did not reach statistical significance ($p = .08$). However, when the proportions of units with durations less than 2.5 ms were compared between injected and noninjected muscles, fewer motor units of short duration were seen in injected muscles ($\chi^2 = 5.37$; $p = .02$). This effect remained significant when only data from patient 4 were examined ($\chi^2 = 5.64$; $p = .02$).

Each motor unit was recorded for at least 10 firings and the standard deviation of the durations was measured among firings for each unit as a measure of motor unit variability. ANOVAs were computed to examine patient, sampling site (anterior versus other), and treatment effects within patients. There was a significant patient effect ($F = 15.69$; $p \leq .0005$) but no significant sample site effect within patients ($F = 0.452$; $p = .867$). When the treatment effects (injected versus noninjected effects) were examined while correcting for patient effects, significant differences were found ($F = 8.897$; $p = .004$). Four patients (Nos. 1, 2, 3, and 7) had greater variability in their injected muscles than in their noninjected muscles (Table 2).

Because the motor unit amplitudes had a skewed distribution, the amplitudes were normalized by multiplying by 1,000. ANOVAs were computed to examine patient effects and sample site effects within patients. No significant patient effects were found ($F = 1.555$; $p = .167$); however, the site effects within patients were significant ($F = 2.909$; $p = .008$). When the treatment effects were examined while correcting for patient and site effects, they were not significant ($F = 2.503$; $p = .16$). When the patient means were examined (Table 2), the treatment effects differed across patients. The amplitudes were greater on the injected side in 3 patients (1, 4, and 6), smaller on the injected side in 3 (2, 3, and 7), and equivalent in 1 (5).

The standard deviations of motor unit amplitudes were also examined with an ANOVA. No significant patient effect was found ($F = .753$; $p = .609$), while a significant sample site effect was found within patients ($F = 2.521$; $p = .019$). The treatment effects were not significant when corrected for site and

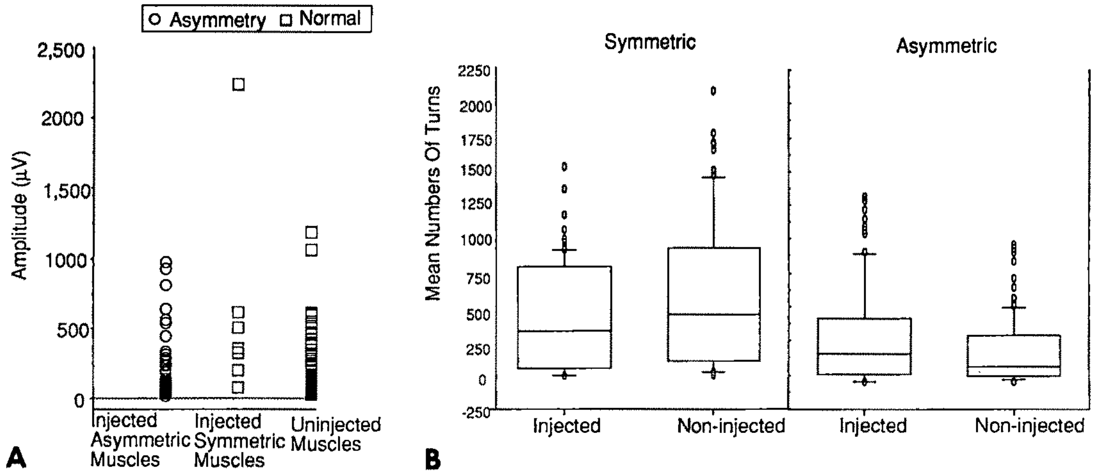

Fig 4. Effects of vocal fold asymmetry on motor unit amplitudes and mean number of turns. A) Scattergram of mean motor unit amplitudes (microvolts) found in injected muscles in asymmetric larynges, injected muscles in symmetric larynges, and noninjected muscles. B) Box plots showing median and quartile distributions for mean numbers of turns per second found in injected and noninjected muscles from symmetric larynges and injected and noninjected muscles from asymmetric larynges.

patient differences (F = .725; p = .396). The amplitude standard deviation was greater in treated muscles in 3 patients, less in treated muscles in 3, and equal in 1.

The distributions of the mean numbers of turns were skewed and the data were normalized by computing the square root of the total numbers of turns measured. The ANOVA results demonstrated significant patient effects (F = 14.914; p ≤ .0005) and nonsignificant site effects within patients (F = 1.520; p = .159). No significant treatment effects were found when the data were corrected for patient and site. However, because the turn data were noticeably different in patient 4, who was sampled with a bipolar electrode, the same ANOVA was computed to exclude this patient. The resulting analysis demonstrated significant treatment effects (F = 9.368; p = .002) after the data were corrected for patient and site. As can be seen in Table 2, however, the mean number of turns was decreased on the injected side in patients 3, 4, 5, and 7 and increased on the injected side in patients 1, 2, and 6. Thus, the treatment effects differed between patients.

Comparisons Between Measures Made from Symmetric and Asymmetric Motion. Motion abnormalities appeared to be related to motor unit amplitudes. Comparison of the motor unit amplitudes from injected muscles in patients having normal (symmetric) motion with those from patients with asymmetric motion revealed greater mean amplitudes in the group with normal motion (F = 8.41; p = .0056). However, these results are somewhat misleading, because only 7 units were found in the injected muscles with symmetric movement, and 1 unit had a very high amplitude (Fig 4A). Whether vocal fold motion was symmetric or asymmetric did not demonstrate any differences, however, in motor unit duration mean or standard deviation (p ≥ .05). None of these results

TABLE 3. SPEARMAN RANK CORRELATION COEFFICIENTS BETWEEN MOTOR UNIT CHARACTERISTICS AND INJECTION CHARACTERISTICS AND PATIENT AGE

Motor Unit Characteristics	*Injection Dosage*	*No. of Injections*	*Time Since Last Injection*	*Age*
Injected muscles				
Amplitude	–.30	.05	.19	–.03
Duration	.09	–.08	–.09	.46*
Duration SD	.35*	.12	–.02	.43*
No. of turns	–.32*	–.03	.30*	.09
Noninjected muscles				
Amplitude		.07	–.09	–.01
Duration		.12	.06	.46*
Duration SD		.09	.09	.51*
No. of turns		–.35*	–.03	.08

*p < .02.

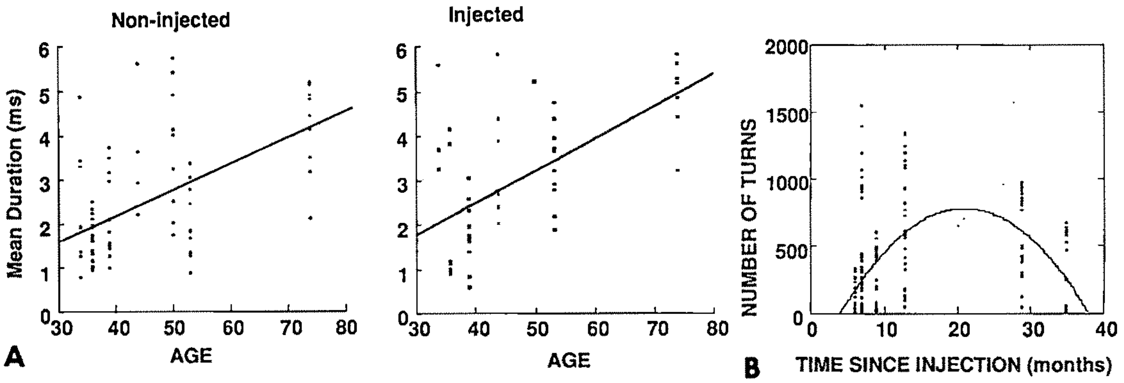

Fig 5. A) Scattergrams demonstrating relationship and line of best fit between mean durations of motor units and patient age (milliseconds) for noninjected and injected muscles. B) Scattergram demonstrating relationship and line of quadratic fit between numbers of turns in injected muscles only and time since patient's last injection in months.

were changed if patient 4 was excluded from the analyses.

Significant differences were found between the mean numbers of turns in muscles sampled from patients with symmetric movement versus patients with asymmetric movement (F = 32.16; p = .0001), and these differences interacted with whether samples came from injected or noninjected muscles (F = 9.804; p = .0019). As shown in Fig 4B, the mean numbers of turns were higher in samples coming from muscles in symmetric larynges when compared to samples from asymmetric larynges, regardless of whether they were from injected or noninjected muscles. This was the case whether or not patient 4 was included in the analysis.

Relationships Between Measures and Injection Characteristics. Other variables were investigated for relationships with motor unit duration, variability, amplitude, and mean numbers of turns. These included total botulinum dose, number of injections, time since last injection, and age (Table 3). Spearman correlation analyses for these variables revealed significant ($p \leq .02$) correlations between total dose and duration variability and mean numbers of turns. The variability of motor units was greater in muscles injected with a higher dosage of botulinum toxin. The numbers of turns in samples were greater in muscles injected with smaller amounts of botulinum toxin or with the greatest amount of time since last injection. The mean numbers of turns were also inversely related to the numbers of injections in noninjected muscles. The duration of motor units and their variability were significantly related to age in both injected and noninjected muscles (Fig 5A), as was reported by Knutsson et al.[34] When the same comparisons were computed after excluding patient 4, similar relationships were found. In addition, a significant relationship was found be-tween the number of turns and age in noninjected muscles (Spearman R = .37; $p \leq .02$) when the data for patient 4 were excluded.

Comparisons Between Samples Made Less Than or Greater Than One Year After Injection. Because of the significant relationship between the mean numbers of turns in samples and time since injection, the data were analyzed to determine if there were differences in motor unit characteristics or mean numbers of turns in patients injected less than or greater than 1 year prior to the EMG recording (Table 4). Significant differences ($p \leq .02$) were found for three variables in the injected samples. The mean durations were longer in units studied within 1 year of injection. Units studied more than a year following injection had higher amplitudes and greater numbers of turns in samples. The same differences were found when the patient 4 data were excluded; the resulting Z scores had p values <.02 on all four comparisons. No significant differences were found in the characteris-

TABLE 4. COMPARISONS BETWEEN MOTOR UNITS IN INJECTED MUSCLES LESS THAN OR MORE THAN ONE YEAR SINCE LAST INJECTION

	Less Than 1 Year			*More Than 1 Year*			*Mann Whitney U*	
Characteristic	*N*	*Mean*	*SD*	*N*	*Mean*	*SD*	*Z*	*p*
Duration (ms)	22	3.97	2.16	29	2.702	1.23	–2.187	.0287
Duration SD	22	1.28	0.85	29	0.988	0.46	–0.913	NS
Amplitude (μV)	22	169	178	29	353.6	446	–2.09	.0364
Mean No. of turns	105	258	320	87	509	387	–4.56	.0001

NS — not significant.

tics of noninjected muscles sampled less than a year or more than a year past injection.

To further address the relationship between time since injection and mean number of turns in the injected muscles, quadratic regression analyses were performed. A significant quadratic relationship was found between the mean number of turns and the time since last injection ($F = 26.120$; $p \leq .0005$). This relationship is plotted in Fig 5B, demonstrating an initial increase in number of turns, rising to a maximum at approximately 20 months and decreasing afterward. A similar trend was found in mean amplitude in the injected muscles ($F = 3.058$; $p = .05$). No significant quadratic relationships were found in the untreated muscles, either for turns or for amplitude.

DISCUSSION

Because no significant differences were found when the data were analyzed with or without patient 4 (sampled with a bipolar concentric electrode, in contrast with the other patients, who were sampled with concentric electrodes), the data from the group as a whole will be discussed. Our findings of primarily biphasic and triphasic units in the laryngeal muscles are similar to previous reports on normal laryngeal muscles by Faaborg-Andersen[35] and Knutsson et al.[34] Only 4 of our 128 units (3%) were polyphasic. Our results are similar to those of Knutsson et al, who reported that 5% of units in normal laryngeal muscles were polyphasic. However, these results contrast with those of Blitzer et al,[6] who reported that 11% of motor units they detected in SD patients were polyphasic. Differences between their patient group and ours might account for this. Two of their patients had already had a recurrent laryngeal nerve crush for treatment of their SD, 3 had other dystonias in addition to SD, 1 had generalized dystonia, and another had received a diagnosis of extrapyramidal and pyramidal disease. Our sample was somewhat more selective; none had had other neurological disorders or recurrent laryngeal nerve surgery, and all only had symptoms of adductor SD.

Our duration measures cannot be compared to those of Faaborg-Andersen[35] or Knutsson et al,[34] because we measured the peak-to-peak durations, a more reliable duration measure[37] than the total duration. The amplitude measurement techniques were comparable, however. The mean motor unit amplitudes measured for units in the noninjected muscles were 194 μV, similar to the most frequent amplitudes of 200 μV reported by Knutsson et al[34] and the range reported by Faaborg-Andersen of between 100 and 300 μV.[35] Overall, then, the mean amplitudes of units from noninjected muscles were similar to normal, suggesting that the SD patients did not have greater amplitudes. Blitzer et al[6] reported that 3 of their SD patients had high-amplitude units; again, this may reflect differences in the patients studied.

This study revealed that repeated botulinum toxin injections were associated with motor unit durations that were longer in injected than in noninjected muscles. This may be explained by the apparent loss of units of shorter duration in injected muscles (Fig 3B). Motor unit duration should be independent of the electrode–motor unit distance and is a reflection of the size of the unit and the synchronicity of firing of the muscle fibers involved. Therefore, our findings may reflect the disorganization of newly reinnervated motor units after administration of botulinum toxin. Synchronicity of neuromuscular end plate discharges may be suboptimal as motor units reform. End plate function may also be less stable in the newly formed motor unit, and this may be a factor contributing to increased duration.

Less than 1 year following injection, the durations were increased in injected muscles (mean, 3.97 ms). The mean duration decreased, however, in patients tested over 1 year since last injection, to a range similar to that of noninjected muscles (2.7 ms). These changes suggest that the time of firing is initially increased, possibly because of collateral sprouting in the end plate region,[26,27] and that this begins to be reduced after 1 year. The finding that the distribution of durations was less when the time since last injection was more than 1 year suggests that either more shorter-duration units became innervated after 1 year, or that the longer-duration units became reduced in number.

The measure of the number of turns is thought to reflect the numbers of motor units firing within a muscle during activation. The mean number of turns in samples from muscles contained in symmetric larynges was greater than that from samples of asymmetric larynges. This difference occurred in both injected and noninjected muscles. Also, the numbers of turns increased dramatically between 6 months and 1 year, then appeared to maximize, and then decreased toward the end of the third year (Fig 5B). Thus, the numbers of motor units innervated after injection continued to increase at 1 year following injection. However, the subsequent decreases in numbers of turns 3 years following injection suggest continuing changes in physiologic function. This might suggest that some of the motor units reinnervated by the end of the first year do not survive and are lost 3 years following injection. Thus, the process of motor unit refinement seems to last up to 3 years after injection. Longer follow-up will be necessary to determine if motor unit characteristics will ultimately normalize.

The mean amplitudes of units from injected muscles were higher (mean, 274 μV) than in noninjected muscles (mean, 194 μV), although not statistically different. However, differences were found between unit amplitudes in muscles studied at different times following treatment, with a similar quadratic relationship with time as was found for numbers of turns in the injected muscles. Units sampled more than 1 year following injection had higher amplitudes than those sampled earlier, while those sampled after 20 months had smaller amplitudes. These physiologic results are in agreement with the histologic changes that have been reported showing that single axons produce sprouts to innervate additional muscle fibers.[26]

The long-term results, however, at 3 years postinjection suggest that some of these sprouts do not remain physiologically active. The data in Fig 5B demonstrate that the numbers of turns decreased after 20 months. Similar significant relationships were found for unit amplitudes over time following injection. Thus, the numbers of larger-amplitude units were decreased. This may be because the collateral sprouts that may have been responsible for the increased amplitudes in the first year may not have survived more than 20 months after injection.

All of the patients with vocal fold asymmetry had received five or more treatments, while those with normal symmetry had received only three or fewer treatments. The finding that the number of turns was reduced in injected and noninjected muscle samples from asymmetric larynges suggests that noninjected muscles may have been affected by the botulinum toxin injections in these patients. Three reports have recently examined differences in symptom control following unilateral and bilateral injections in adductor SD.[19,20,38] All three reported that the degree and duration of symptom control was somewhat greater following unilateral injections in comparison with bilateral injections. Perhaps the more pronounced physiologic effects following unilateral injections resulting in movement asymmetries could account for the somewhat greater symptom control following unilateral injections.

Association of motion abnormality with an increased number of injections, and not with the total dose, leads us to speculate that repeated botulinum toxin injections, each one requiring needle electrode insertions, causes alterations in vocal fold motion. Some of these patients appear to have developed vocal fold shortening and decreased abduction on the side of injection. This may have resulted from inflammation and fibrosis following repeated injuries, leading to mechanical changes in the TA muscle preventing lengthening and full abduction. An alternative explanation may be diffusion of botulinum toxin at the time of injection to associated laryngeal muscles. Long-term motion characteristics would then be dependent on the reinnervation patterns of these muscles as well as the TA. If diffusion is a significant factor in the development of motor abnormalities after botulinum injections, then the current technique of lower-dose bilateral injections should be associated with fewer motion alterations. However, if motion abnormalities result from repeated needle trauma, patients injected repeatedly with the bilateral technique might develop bilateral movement alterations.

Similar significant relationships were found between motor unit duration and age in both injected and noninjected muscles (Fig 5A). Knutsson et al[34] reported a small but statistically significant difference in motor unit duration with age. They reported that the mean duration of motor units in their older subjects (40 to 60 years) was 4.21 ms, which was longer than in their younger group (20 to 30 years), who had a mean duration of 3.43 ms. Similar increases in motor unit duration with age have been reported in other muscles in the body,[39-41] although the slopes usually are less steep than those found in our SD patients. The effects of aging may have been exacerbated either by botulinum toxin injection or by the motor control disorder in SD patients. These results suggest that further careful measures of motor unit physiology need to be conducted in these patients.

In conclusion, repeated use of botulinum toxin injections in adductor SD was associated with asymmetric vocal fold motion in some patients, and asymmetry may be related to the number of injections. An increase in motor unit duration was found that was less significant in patients who were studied over 1 year since their last injection. Motor unit amplitudes and the numbers of turns were increased in patients over 1 year after injection, but were decreased at 3 years postinjection. This may indicate that motor unit reorganization and refinement continues over a year after chemical denervation by botulinum toxin.

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VERTICAL PLANE SHORT AND MIDDLE LATENCY VESTIBULAR EVOKED POTENTIALS IN HUMANS

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In order to determine whether short and middle latency vestibular evoked potentials (VsEPs) can be recorded in humans in response to angular acceleration stimuli in the vertical plane, a drum, head-holder, and stepper motor were designed to deliver upward acceleration impulses of 10,000°/s^2 (1.8° displacement) to the human head. Forehead and mastoid electrodes recorded electrical activity that was filtered, differentially amplified, and averaged in short (12.7 milliseconds) and middle (63.5 milliseconds) latency time frames. Control recordings were used to eliminate various types of artifact. Recordings were conducted in 7 normal subjects and in 4 control patients with congenital, profound hearing loss and absence of caloric responses. Short and middle latency VsEPs with high intrasubject and intersubject consistency were recorded in normal subjects and not in control patients. The middle latency responses were larger in amplitude than the short latency responses. The effects of stimulus intensity and repetition rate on VsEP waveform, latency, and amplitude were studied. Experiments have shown that the responses are not electrical artifact, nor are they contaminated by auditory, somatosensory, or passive eye movement potentials.

KEY WORDS — acceleration, evoked potentials, short latency, vestibular system.

INTRODUCTION

There have been very few studies devoted to the recording of vestibular evoked potentials (VsEPs) to acceleration impulse stimuli. This paucity is due to the need to deliver stimuli of high intensity with sufficiently rapid rise times to synchronously activate a large number of vestibular neurons. In addition, these stimuli must be exactly replicable on repeated stimulation in order to average the responses to them. Our group has succeeded in achieving this with angular acceleration impulses in the horizontal plane (stimulation of the lateral semicircular canal [SCC]) leading to the recording of short latency VsEPs in rats,[1] cats,[2] and humans.[3,4]

In order to complement the horizontal plane angular acceleration VsEPs, a new drum was designed and constructed to deliver angular acceleration impulses in the vertical plane (pitch stimuli), since stimulation in this plane would be more conducive to routine clinical use. Following initial experiments on rats and cats that showed the feasibility of such stimulation and allowed control for possible artifacts, the same stimulus system was used to deliver impulses of angular acceleration to the human head. This report presents the results of such experiments on humans in the short and middle latency time frames, arbitrarily chosen as 12.7 and 63.5 milliseconds (ms), respectively.

METHODS

Stimulus (Patented). The same stepper motor used in the earlier study for delivering angular acceleration stimuli in the horizontal plane[3,4] in humans was incorporated into this new system. The new drum delivered repeated impulses of angular acceleration in the vertical plane through the interaural axis (pitch). An impression of the teeth of the upper jaw was taken from each subject, and an individually pressed acrylic dental plate was molded from it. The subject lay supine on a bed facing straight up with his or her head held flexed 20° to 25° with respect to the long axis of the body. The dental plate, gripping the teeth, was attached to the rim of the drum by an acrylic bar. With each stimulus, the nose was tilted upward 1.8° (see diagram in Fig 1) with a maximal acceleration of 10,000°/s^2, and a rise time of about 3 ms (a slope of about 3,000°/s^2 per millisecond). This was followed by a return step of lower acceleration in order to return the head to the starting position. This stimulus was usually delivered at the rate of 1/s, at which time the return step came after 500 ms. The stimulus could also be delivered at 0.5/s and 2/s. The acceleration stimuli were measured with a Bruel and Kjaer 4366 accelerometer on the acrylic bar close to the dental

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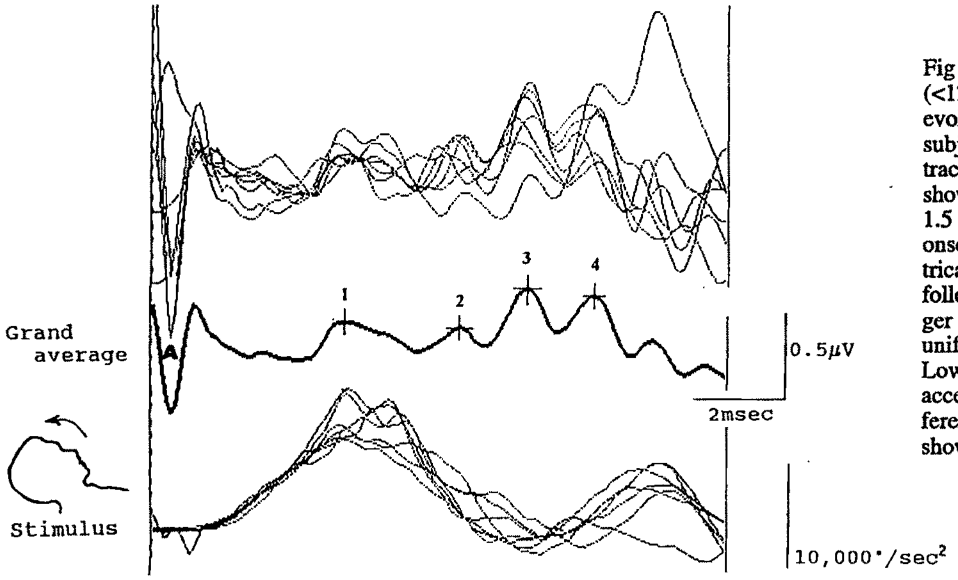

Fig 1. Upper traces show short latency (<12.7 milliseconds [ms]) vestibular evoked potentials (VsEPs) in normal subject over 13-month period. Middle trace shows grand average of all traces shown above, with peaks labeled. First 1.5 ms of electrical activity (before onset of acceleration stimulus) is electrical artifact (marked A on this and following relevant Figures) due to trigger pulse to motor (waveform not uniform on different recording days). Lower traces show average (N = 32) acceleration pulse waveforms on different recording days. Diagram at left shows direction of head rotation.

plate. Single acceleration impulse waveforms were identical to averaged (N = 32) acceleration pulses, indicating their replicability.

Recording. Disposable pediatric electrocardiogram electrodes were applied to both mastoids and the forehead. The recordings were conducted as the potential difference between the forehead and the left mastoid. (The other mastoid served as a ground.) A Microshev 2000 evoked response system was used. The recorded electrical activity was digitally filtered between 300 and 1,500 Hz for the short latency window and between 30 and 300 Hz for the middle latency window. These were found to be optimal following experimentation with the various band passes available.[5] Each response trace consisted of the average of 256 or 512 responses (short latency) and 64 or 128 responses (middle latency). Several recordings were conducted in each session to determine response repeatability, and grand averages of all of the traces obtained were generated. The responses were displayed forehead positive up.

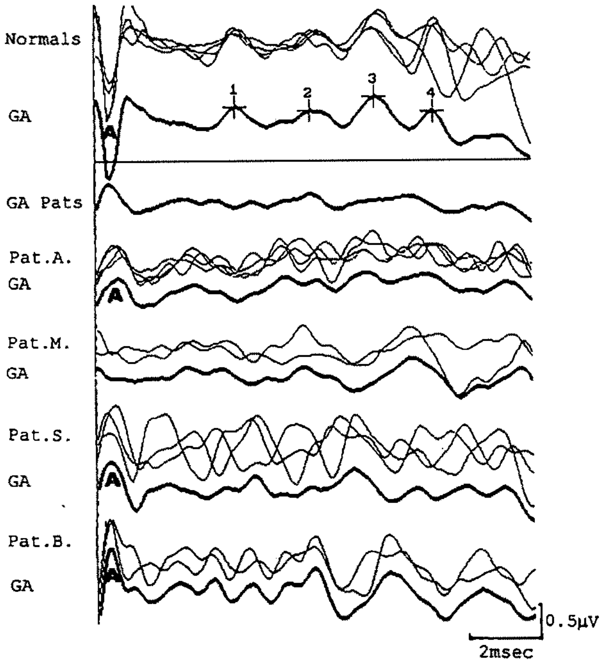

Fig 2. Upper traces show grand average (GA) short latency VsEPs over all recording sessions from 4 normal subjects and GA from these subjects; note four clear and consistent waves. Below is GA of all short latency activity from 4 control patients (GA Pats; bilateral profound hearing loss, absence of caloric electronystagmography responses) and repeated averaged responses and GA from each patient.

Subjects. Seven normal subjects participated in the study. There were 5 males and 2 females, 17 to 20 years old, with normal hearing. In addition, 4 patients (3 females and 1 male), 19 to 29 years old, with bilateral profound hearing loss and absence of electronystagmographic responses in bithermal caloric tests served as controls. Otherwise, they were normal. The Committee for Human Experimentation approved the experimental protocol, and informed consent was obtained from each subject or his or her guardian.

RESULTS

Short Latency VsEPs. The short latency (12.7 ms window) VsEPs in humans consisted of several waves. The onset of the first clear wave is at about 2.2 ms after the onset of the acceleration impulse, with a forehead positive peak at 2.9 ms after acceleration onset. This is followed by an additional three clear waves with peak latencies of 5.1, 7.0, and 8.6 ms after the onset of the acceleration stimulus, with amplitudes of 0.3 to 0.6 μV. The VsEPs in human subjects were unchanged, even following 2 hours of almost continuous stimulation (Figs 1 and 2).

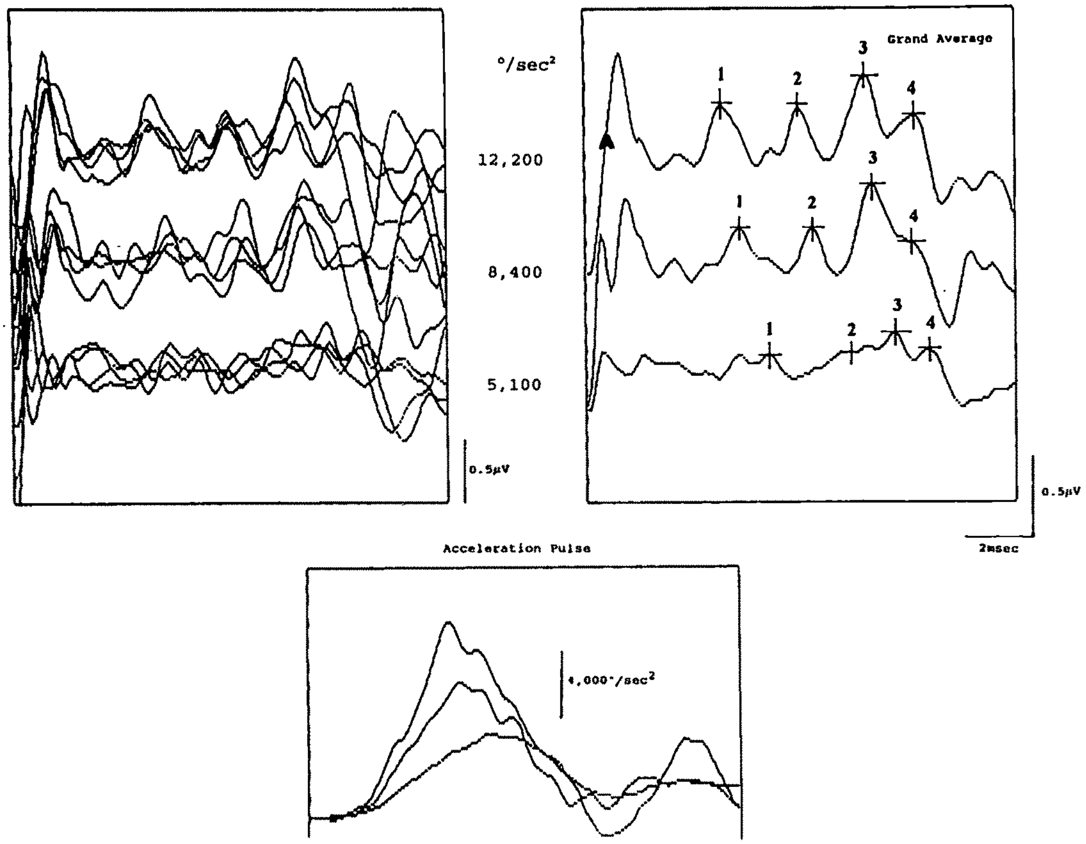

Fig 3. Effects of three different peak intensities of angular acceleration impulses in vertical plane in 4 normal subjects. Note that as stimulus intensity is decreased, VsEP amplitudes decrease and latencies increase.

In the 4 control patients with bilateral profound hearing loss and absence of caloric responses, coherent, consistent responses could not be demonstrated, except perhaps for a small response, differing in waveform, in patient B (Fig 2). The sounds that accompanied drum movement were measured with a Bruel and Kjaer microphone and octave filter set. They began with lower intensities and higher fre-

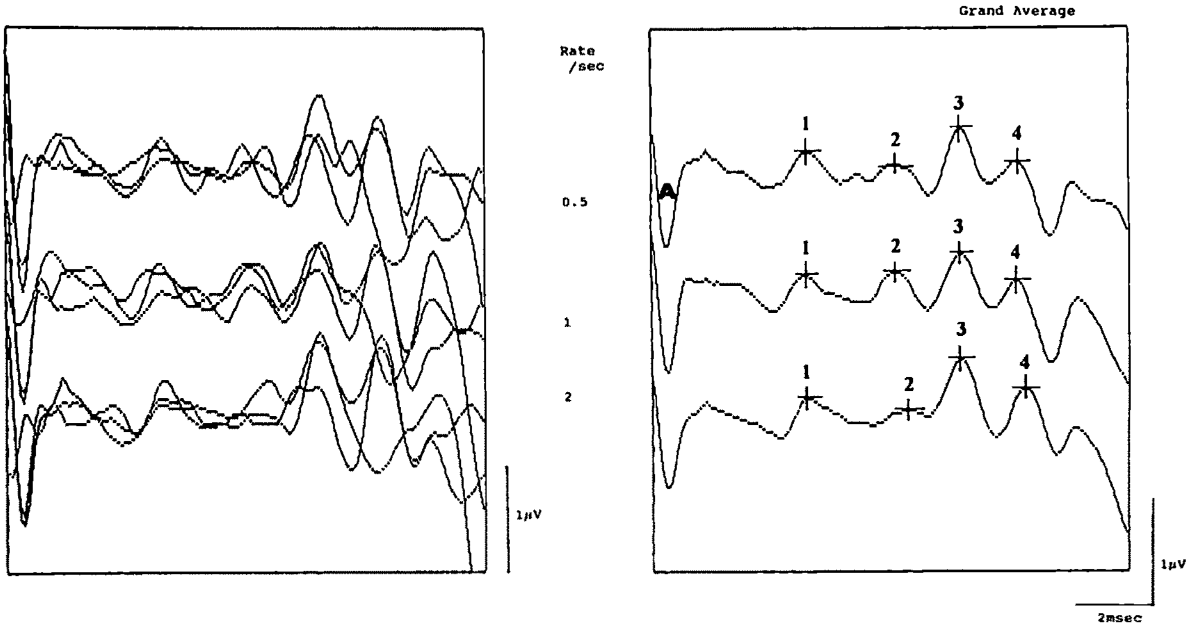

Fig 4. Effects of three different stimulus rates on short latency VsEPs in 3 normal subjects. These stimulation rates (0.5/s, 1/s, and 2/s) did not affect VsEPs.

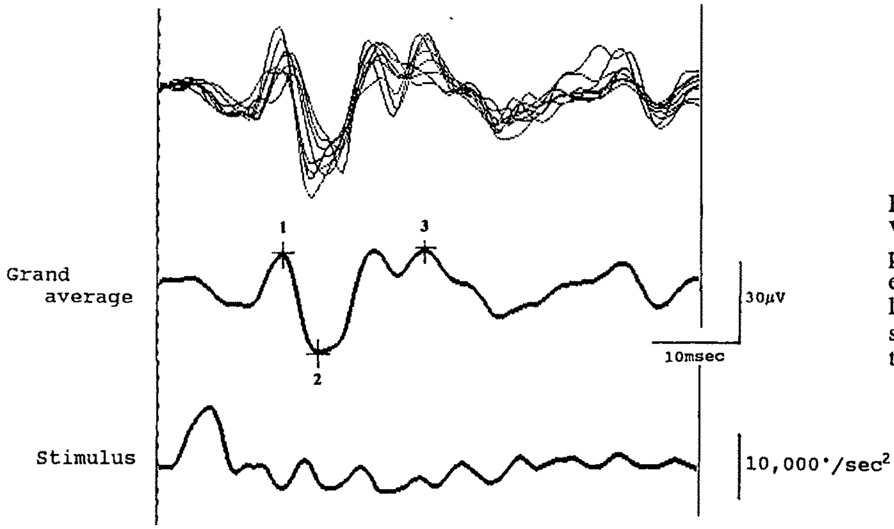

Fig 5. Upper traces show middle latency VsEPs in normal subject over 6-month period. Middle trace shows grand average of above responses, with waves labeled. Lowest trace shows averaged stimulus waveform. Note that acceleration was over before onset of response.

quencies simultaneous with the rise of the acceleration, reaching a low frequency (around 63 Hz) and higher intensity (82 dB peak equivalent sound pressure level) after 20 ms. A sound with such a slow rise time to a low-frequency peak is not likely to elicit auditory evoked potentials. Possible auditory response contamination was further ruled out when the VsEPs were shown to be unaffected by 100 dB sound pressure level white noise masking (which would also mask bone conducted sounds) and by attenuating earplugs. Finally, when the subject's head was not in the moving, stimulating drum, a coherent response could not be recorded.

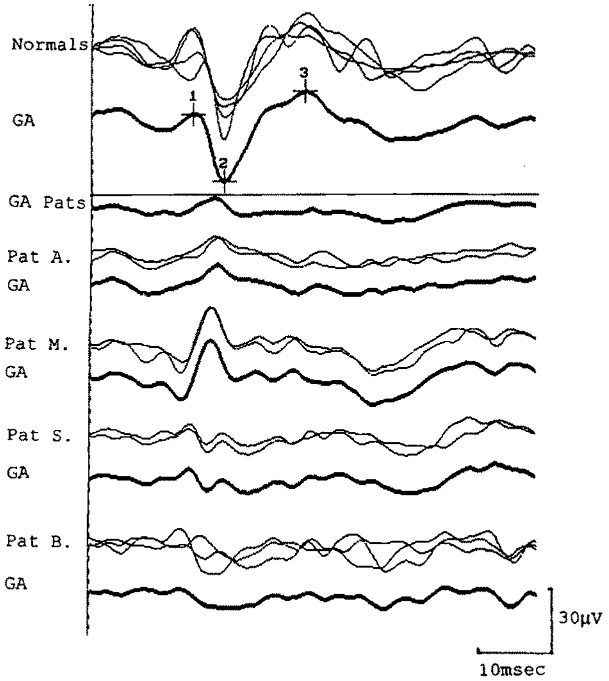

Fig 6. Upper traces show middle latency VsEPs in 4 normal subjects and their grand average (GA). Lower traces show GA of all middle latency activity recorded in 4 control patients (GA Pats; absence of caloric responses) and repeated average responses and GA from each patient. Note absence of consistent response in patients.

The effects of different intensities and rates of stimulation on the VsEPs were also studied in 4 normal subjects. As stimulus intensity was decreased, the amplitudes can be seen to decrease and the latency to increase (Fig 3). The rate of stimulation in the range of 0.5/s to 2.0/s, however, did not affect the latencies and amplitudes of the VsEPs (Fig 4).

Middle Latency VsEPs. Similar series of experiments were conducted in the middle latency (63.5 ms) time frame. The middle latency VsEP is dominated by a forehead positive peak at 14.5 ms and a negative peak at 19.0 ms with large amplitudes: 27 μV peak to peak. There is a smaller positive peak at 31.0 ms (Fig 5). In the patients with bilateral absence of caloric responses, the response is not consistent, very small in amplitude (patient S), or of opposite phase (patient M) with respect to the responses in normal subjects (Fig 6).

DISCUSSION

Following the initial successful feasibility studies in experimental animals, this study has shown that short and middle latency VsEP responses to angular acceleration impulses in the vertical plane can be recorded in human subjects also. This was achieved only after modification of the stimulus system to deliver impulses of angular acceleration with rapid rise times (reaching 10,000°/s^2 within 3 ms) and with high replicability in the vertical plane.

The small displacement (a total of 1.8°) and short duration of the stimulus (followed by a return step) may explain the absence of complaints of dizziness, pain, or any other discomfort by the subjects and their ability to fall asleep during the recording session.

Rigorous control procedures were conducted. Chief among these were attempts to record VsEPs in patients with bilateral profound hearing loss and absence of electronystagmographic responses to bithermal caloric stimuli. This indicates loss of response to low-frequency stimuli,[6] chiefly from the lateral SCCs. Otherwise, these patients were normal. Consistent VsEP responses could not be seen in these patients. On the other hand, in the normal subjects, there was great intrasubject and intersubject consistency in the VsEPs. This showed that the ability to record short and middle latency VsEPs in the vertical plane requires normal vestibular function and rules out electric and passive eye movement artifact and contamination from somatosensory and visual responses. An early (less than 1.5 ms), inconsistent electrical artifact was present even before the onset of the acceleration stimulus, and it was over before the onset of VsEP responses, so it did not contaminate the response. The onset of the first VsEP response wave occurs before the peak of the acceleration stimulus. Therefore, initiation of neural activity in the end organ probably begins sometime during the rising phase of the acceleration impulse.

Other control procedures ruled out contributions from auditory and somatosensory responses. These are similar to the controls used when recording the VsEPs to horizontal plane stimulation.[3,4] The VsEPs to stimulation in both planes studied (horizontal and vertical) are similar in latency and amplitude, and those in the middle latency time frame (both horizontal and vertical planes) are consistently larger in amplitude (about 50 times) than the shorter latency.

The short latency VsEPs were affected by the intensity of angular acceleration stimuli, with amplitudes increasing and latencies decreasing as stimulus intensity was increased. On the other hand, changes in stimulus rate between 0.5/s and 2/s were without effect. Higher stimulation rates were not feasible, since they were uncomfortable for the subject. This is similar to the effects of such ranges of stimulus intensities and rates on the short latency auditory evoked potentials (auditory nerve brain stem evoked responses).[7]

The short latency VsEPs are made up of several scalp positive waves, and qualitatively similar waves in response to identical stimuli have been recorded in cats. In cats it has been shown that the VsEPs are initiated in the SCC optimally stimulated[8] and that the first wave is generated in the vestibular nerve. The second wave is generated in the region of the vestibular nucleus.[9] Recent experimentation in this laboratory has provided evidence that the succeeding waves may derive from activity in the motor nuclei of the cranial nerves innervating the extraocular muscles.

In the present experiments, angular acceleration impulses were delivered to the head in the interaural axis, tilting the nose upward. Given the anatomy of the three SCCs[10] and the angle of the head during stimulation, this mode and plane of stimulation would be excitatory to both (left and right) posterior SCCs, since it produces utriculofugal endolymph flow in these canals. Other SCCs and the otolith organs may also be stimulated, although to a smaller degree. In order to achieve optimal stimulation of a single posterior SCC — for example, the right — the head would have to be tilted 55° to the right with respect to the long axis of the body (yaw) and 20° with respect to the naso-occipital axis (left ear up; roll) with upward accelerations then delivered.[10] The VsEPs to such stimuli will be investigated in the future.

The source(s) of the middle latency VsEPs are not clear as yet. As deduced from the effects in cats of muscle relaxant drugs on some of these waves,[2] there may be myogenic contributions. Further experimentation is required to determine the sites of electrical activity leading to the middle latency VsEP.

There is reason to believe that stimulation in the vertical plane will be more convenient than horizontal for routine clinical use, since it may be possible to stimulate in this plane with less rigorous gripping of the teeth. Furthermore, following clarification of the effect of head angle on these responses, it may be possible to differentially record responses initiated in each of the SCCs.

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ROLE OF HERPES SIMPLEX VIRUS INFECTION IN THE PATHOGENESIS OF FACIAL PARALYSIS IN MICE

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To clarify the role and site of herpes simplex virus (HSV) infection in the pathogenesis of facial paralysis, we examined the viral genome by the polymerase chain reaction and the neutralization antibody titer using microplates in an animal model. Following inoculation with HSV type 1 of the KOS strain into mouse auricles, HSV DNA appeared in the ipsilateral facial nerve on the 3rd day, and in bilateral facial nerves and the brain stem on the 10th day only in animals with facial paralysis. In animals without facial paralysis, no HSV DNA was detected in these tissues. The neutralization antibody titer was elevated between 4 and 20 days in all animals, with or without facial paralysis. Facial paralysis developed only on the inoculated side, even though HSV DNA was also present in the contralateral facial nerve. We conclude that HSV infection in the facial nerve and brain stem is prerequisite for facial paralysis, and suggest that an immunologic reaction following viral infection plays a key role in the pathogenesis.

KEY WORDS — facial paralysis, herpes simplex virus, neutralization antibody, polymerase chain reaction, viral migration.

INTRODUCTION

It has been suspected that herpes simplex virus (HSV) is a causative agent of Bell's palsy. Several animal experiments have been conducted to prove HSV infection as a cause of facial paralysis by inoculating it into the facial nerve,[1] tongue,[2,3] nasal mucosa,[2] or auricle,[4,5] but the findings have been inconsistent. Moreover, no agreement was obtained as to the pathogenesis of facial paralysis. In our animal model with facial paralysis, HSV antigen was not always detectable with indirect immunofluorescence and the avidin-biotin peroxidase complex.[5] To the contrary, facial paralysis did not develop even if HSV antigen was present in the facial nerve and its nucleus in other animal models.[2,3] So far, the role of HSV infection in the pathogenesis of facial paralysis has remained unclear.

Here we applied polymerase chain reaction (PCR) analysis to our animal model of an acute and transient facial paralysis induced by HSV infection[5] and traced the migration route of the virus in the craniofacial neural system. We also examined neutralization antibody titers to HSV at various intervals postinoculation to elucidate the relationship between viral infection and facial paralysis.

MATERIALS AND METHODS

Animals and Virus Inoculation. Forty-four 4-week-old female Balb/c mice were studied. Twenty-four were analyzed by PCR, and 20 were studied serologically. All animals were maintained in our Laboratory Animal Center, and studies proceeded in compliance with the Guide for Animal Experimentation at Ehime University School of Medicine. After scratching the surface of the bilateral auricles by a 27-gauge needle, 25 μL of the KOS strain of HSV type 1 with a titer of 4.5×10^6 plaque-forming units per milliliter was inoculated into the right auricle, and the same volume of phosphate-buffered saline (PBS) was placed in the left. As a control, PBS was placed on the bilateral auricles of 4 mice. In a previous study, facial nerve paralysis developed only on the side inoculated with the virus, between 6 and 9 days thereafter, and continued for 3 to 7 days.[5]

Samples for PCR. Twenty-four animals were divided into six groups, each consisting of 4 animals: 1) control; 2) group 1, 3 days postinoculation; 3) group 2, 10 days postinoculation with facial paralysis; 4) group 3, 10 days postinoculation without facial paralysis; 5) group 4, 24 days postinoculation; and 6) group 5, 90 days postinoculation. All the animals in groups 4 and 5 developed facial paralysis and recovered completely by the time samples were obtained. Following general anesthesia with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), animals were painlessly sacrificed by transcardial perfusion with 50 mL of saline. Thereafter, bilateral intra-

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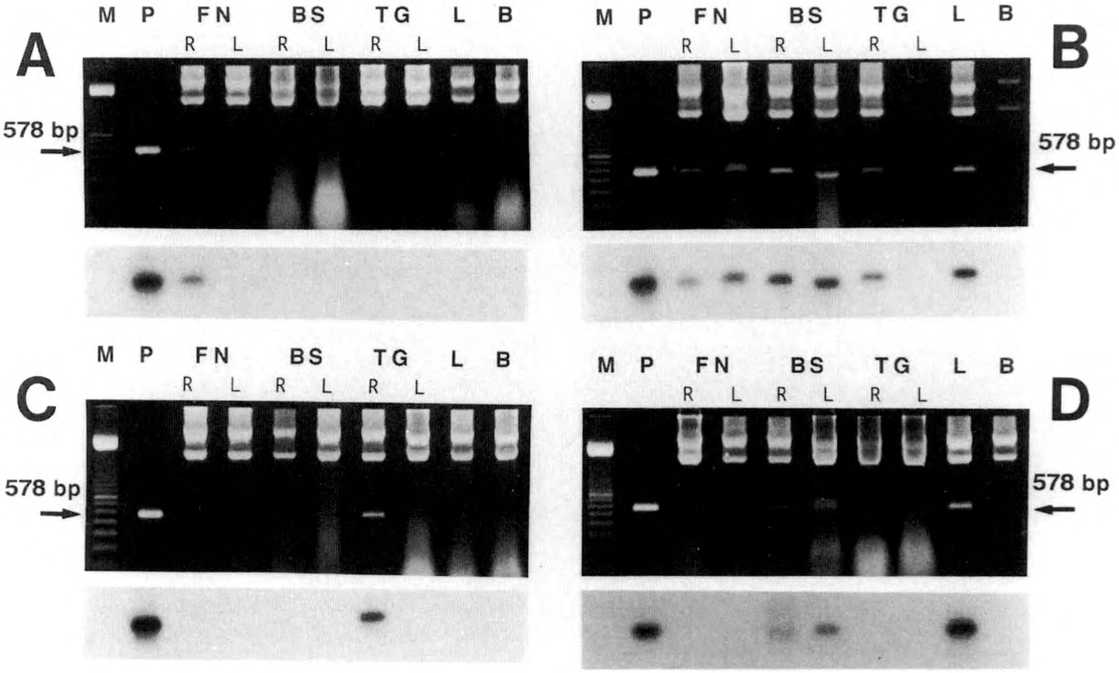

Fig 1. Detection of herpes simplex virus (HSV) DNA in facial nerve (FN), brain stem (BS), trigeminal ganglion (TG), cerebrospinal fluid (CSF; L), and blood (B) by polymerase chain reaction. Upper panels indicate ethidium bromide staining, and lower panels, Southern blot hybridization. M—molecular size marker (100 base pair ladder), P—positive control. A) Three days postinoculation. B) Ten days postinoculation with facial paralysis. C) Ten days postinoculation without facial paralysis. D) Ninety days postinoculation. HSV DNA was detected only in FN of inoculated side or CSF on third day postinoculation (A). It became detectable in bilateral FN, BS, TG of inoculated side, and CSF in animals with facial paralysis (B); however, HSV DNA was undetectable in either FN or BS in animals without facial paralysis (C). At 90 days postinoculation, HSV DNA was detectable in BS or CSF, although animals had recovered from facial paralysis (D).

temporal facial nerves, including the geniculate ganglia, brain stem involving the facial motor nucleus, and trigeminal ganglia, were dissected and washed three times with saline. Twenty microliters of peripheral blood was taken before transcardial perfusion, and the same volume of cerebrospinal fluid (CSF) was aspirated from the cisterna magna after the transcardial perfusion.

PCR. The facial nerves, brain stems, and trigeminal ganglia were cut with scissors and added to 100 μL of TE (10 mmol/L Tris-hydrochloride, pH 7.5, and 1 mmol/L ethylenediaminetetraacetic acid [EDTA]). The CSF and blood were diluted with 100 μL of TE. These samples were digested with 20 μg of proteinase K at 56°C for 120 minutes. After heat inactivation of the proteinase K, 2 μg of plasmid DNA (Bluescript KS) carrier was added to the mixture. DNAs was extracted with phenol-chloroform and chloroform, and precipitated by ethanol. The DNAs was dissolved in 5 μL of TE and used as PCR template.

Synthetic primers that are specific for HSV and encode virion glycoprotein C and a hydrophobic N terminus located on the HSV gene UL44 and UL45 (sense, 5′CCACCGAGCGGCAGGTGATC-3′; antisense, 5′-GCCGACCGCCTGCTCGTGCT-3′) were used for PCR amplification under conditions modified as described.[6,7] Briefly, 5 μL of the extracted DNA and two primers (25 pmol each) in 50 μL PCR cocktail (5 μL 10× PCR buffer [670 mmol/L Tris-hydrochloride (pH 8.8), 67 mmol/L magnesium chloride, 1.7 mg/mL bovine serum albumin, 166 mmol/L $(NH_4)_2SO_4$], 5 μL dimethylsulfoxide, 5 μL 10× deoxynucleoside triphosphate [15 mmol/L each], and 30 μL water) were denatured at 95°C for 7 minutes. Native Taq DNA polymerase (2.5 U; Perkin-Elmer-Cetus, Norwalk, Conn) was added and the mixture was overlaid with mineral oil at 95°C. The first annealing and extension reactions were performed at 60°C for 2.5 minutes and 73°C for 40 minutes, respectively. After the initial reaction, 35 cycles of amplification (95°C, 80 seconds; 60°C, 90 seconds; 73°C, 60 seconds) were performed and followed at 73°C by a 15-minute final extension.

Five microliters of PCR products were separated by electrophoresis in Tris-borate-EDTA buffer (1.5%

RESULTS OF POLYMERASE CHAIN REACTION FOR DETECTION OF HERPES SIMPLEX VIRUS DNA

			No. of Virus DNA–Positive Specimens							
			Facial Nerve		*Brain Stem*		*Trigeminal Ganglion*			
	Days Post-inoculation	*Facial Paralysis*	*R*	*L*	*R*	*L*	*R*	*L*	*Cerebrospinal Fluid*	*Blood*
Control		Absent	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Group 1	3	Absent	2/4	0/4	0/4	0/4	1/4	0/4	2/4	0/4
Group 2	10	Present	4/4	3/4	4/4	4/4	2/4	0/4	4/4	0/4
Group 3	10	Absent	0/4	0/4	0/4	0/4	1/4	1/4	1/4	0/4
Group 4	24	Recovered	1/4	0/4	1/4	2/4	0/4	0/4	1/4	0/4
Group 5	90	Recovered	1/4	0/4	1/4	2/4	1/4	0/4	1/4	0/4

Each group consists of 4 animals.

agarose gel containing 0.5 mg/mL ethidium bromide), transferred to Gene Screen Plus (New England Nuclear, Boston, Mass), and hybridized with a ^{32}P-labeled internal oligonucleotide probe. Herpes simplex virus PCR products for Southern blot were detected by means of the internal oligonucleotide sequences located on HSV gene UL44 (5′-GCACATCACAGTCGCGGCAG-3′).

Serum Antibody Titer to HSV. Five microliters of blood was taken from the infraorbital plexus of 20 animals on the 3rd, 10th, and 20th days postinoculation. These sera were stored at −20°C until using. The antibody titers to HSV were assayed by means of the neutralization test using microplates, which is generally believed to be more specific and sensitive for the diagnosis of viral infection.

RESULTS

PCR. Figure 1 shows the PCR analysis of representative animals in each group, and the Table summarizes the data obtained from all the animals. On the 3rd day postinoculation, HSV DNA was detected from 2 of 4 animals in the facial nerve of the inoculated side and CSF (group 1). On the 10th day, HSV DNA was detected not only in the facial nerve, brain stem of the inoculated side, and CSF, but also from the other side of the facial nerve and brain stem in animals with facial paralysis (group 2). On the other hand, HSV DNA could not be amplified from either facial nerve or brain stem in the animals without facial paralysis, although it was amplified from the trigeminal ganglion or CSF (group 3). On the 24th day (group 4), HSV DNA was detectable in the facial nerve, brain stem, or CSF in some animals, even though the facial paralysis had disappeared. Herpes simplex virus DNA was detectable in these tissues up to the 90th day postinoculation (group 5), even though it was difficult to tell by PCR whether the viral genomes represented productive or latently infected virus.

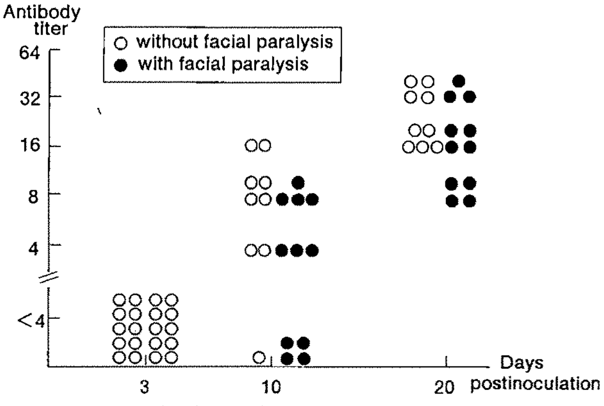

Fig 2. Neutralization antibody titers at 3, 10, and 20 days postinoculation. Neutralization antibody titers were negative (<4) in all animals before 3rd day postinoculation. At 10 days postinoculation, 15 of 20 animals (75%) had positive (≥4) antibody titer, among which 9 developed facial paralysis. All animals underwent complete seroconversion, with titer ranging from 8-fold to 32-fold dilution by 20th day postinoculation.

Herpes simplex virus DNA was not amplified in the blood samples or any other samples obtained from control animals.

Antibody Titer to HSV. The neutralization antibody titers at various periods are shown in Fig 2. The antibody titers were negative in all animals before the 3rd day postinoculation. They became positive at the 10th day, and all animals had undergone complete seroconversion by the 20th day postinoculation. There was no significant difference between the antibody titers of animals with and without facial paralysis.

DISCUSSION

Herpes simplex virus is a neurovirulent virus that is transmitted intra-axonally to the neuron or satellite cells in the ganglion and nucleus.[8,9] We produced an acute and transient facial nerve paralysis by inoculating HSV type 1 in the auricles of mice. However, paralysis developed in only 56% of the animals, and HSV antigen was detected in the facial nerve in about

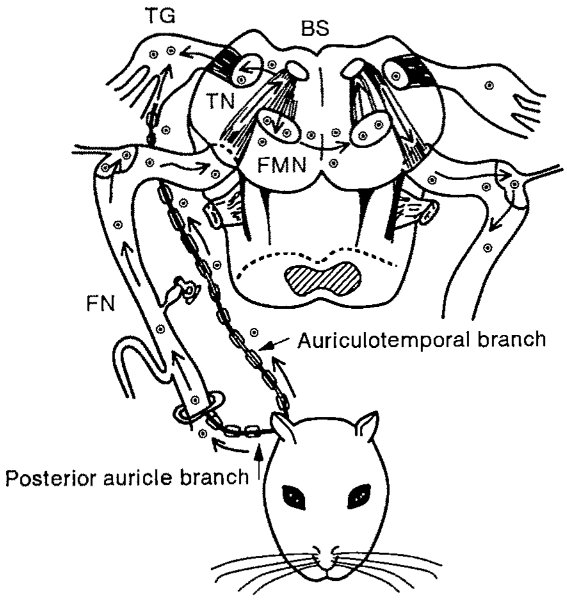

Fig 3. Schematic representation of possible migration pathways of HSV. HSV inoculated in auricle ascends intra-axonally from posterior auricular branch in facial nerve (FN; route 1) or from auriculotemporal branch of trigeminal nerve (route 2) to brain stem (BS) and trigeminal ganglion (TG). HSV widely spreads in BS and migrates to other side of FN or TG. FMN — facial motor nucleus, TN — trigeminal nucleus.

30% of those by indirect immunofluorescence and with the avidin-biotin peroxidase complex.[5] Thomander et al[3] have inoculated two different neuropathogenetic strains of HSV-1 (KJ 502, F) into the tongues of mice and demonstrated viral antigens in 27.8% of the facial nerves, 55.6% of the trigeminal nerves, and 84.2% of the brain stem using peroxidase-antiperoxidase. Following the inoculation of HSV into the tongues of the guinea pigs, Ishii et al[2] also demonstrated HSV antigens in 21% of the facial nerves and 38% of the pons by indirect immunofluorescence and indirect immunoperoxidase. However, these animal models did not develop facial nerve paralysis. The discrepancy between viral infection and the pathogenesis of facial paralysis might be attributed to the limited sensitivity of the classic immunocytochemical methods, or to a difference in animal resistance against HSV infection. The PCR is much more sensitive and clarified not only the role of viral infection in the pathogenesis of facial paralysis, but also the viral migration route from the auricle to the craniofacial neural system in our animal model.

Herpes simplex virus DNA became detectable in the bilateral facial nerve, brain stem, and trigeminal ganglion after 10 days postinoculation in animals with facial paralysis, while it was detectable only in the homolateral facial nerve or trigeminal ganglion after 3 days postinoculation. Based on the PCR results, the migration route of HSV can be traced as follows. Virus inoculated in the auricle first infected the homolateral facial nerve and ascended intra-axonally to the brain stem. Virus spread in the brain stem and migrated to the other side of the facial nerve. Herpes simplex virus DNA was also detected from the trigeminal ganglion or CSF in animals with or without facial paralysis. There are two possible pathways of virus migration from the auricle to the trigeminal ganglion: one is directly through the auriculotemporal branch of the trigeminal nerve, and the other is indirectly via the facial nerve to the brain stem, then to the trigeminal ganglion (Fig 3). The fact that HSV DNA was detectable in the CSF but not in the blood indicated that HSV can cause meningitis without viremia.

On the other hand, HSV DNA was not detected in either the facial nerve or the brain stem of animals without facial paralysis. The neutralization antibody titer was elevated in all animals regardless of whether they had facial paralysis. We thus concluded that HSV infection in the facial nerve and brain stem is a prerequisite and that the development of facial paralysis may depend on whether or not HSV infects the facial nerve.

The next question arises as to why the facial nerve paralysis developed only on the side of virus inoculation, even though HSV DNA was detectable in the other side of the facial nerve and brain stem. Among viral diseases, similar unilaterality is often seen in Ramsay Hunt syndrome and deafness due to mumps virus. Bell's palsy, too, generally shows unilateral facial paralysis, and bilateral paralysis in only 0.3%[10] to 1.2%[11] of patients. However, Safman[12] has noted subclinical involvement in the clinically unaffected facial nerve in 14 of 18 (78%) patients with Bell's palsy by electromyography. Moreover, gadolinium-enhanced magnetic resonance imaging sometimes demonstrates contrast enhancement in the facial nerve of the clinically unaffected side, suggesting subclinical involvement.[13] Our animal model may provide a clue for the role of viral infections in such bilateral involvement with unilateral clinical manifestations. We speculate that the mechanism of unilateral facial paralysis in the animal model is as follows. It required more than 3 days for the virus to reach the uninoculated side of the brain stem and facial nerve as demonstrated by PCR, and the neutralization antibody titer became positive between 4 and 20 days postinoculation. This means that when HSV reaches the uninoculated side of the brain stem or facial nerve, the immune mechanism, including the neutralization antibody, is already activated. Consequently, the virus may not be able to propagate or produce neuritis causing facial paralysis. If sufficient amounts of virus simultaneously infect the facial nerve bilaterally, bilateral facial paralysis would result. In acute and primary herpes infection, agents of cellular immunity such as macrophages,[14,15] natural killer cells, and T cells[16] may react more promptly and play a more important protective role than those of humoral immunity, such as neutralization antibody. Although the immunologic reaction underlying this animal model cannot be fully understood only by the neutralization antibody titer, the timing of viral infection and the subsequent immunologic reaction must play a role in the pathogenesis of facial paralysis.

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MIDDLE EAR MECHANICS IN RESEARCH AND OTOSURGERY

The international workshop Middle Ear Mechanics in Research and Otosurgery will be held in Dresden, Germany, September 19-22, 1996. For further information, contact Prof Dr K. B. Hüttenbrink, Universitäts-HNO-Klinik, Fetscherstr 74, 01307 Dresden, Germany; telephone (Germany) (0)351-458-2224; fax (0)351-458-4326.

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COMPARATIVE PERILYMPH PERMEABILITY OF CEPHALOSPORINS AND ITS SIGNIFICANCE IN THE TREATMENT AND PREVENTION OF SUPPURATIVE LABYRINTHITIS

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Cephalosporins are nonototoxic antibiotics that provide excellent coverage for almost all bacteria that can cause suppurative labyrinthitis. In this study we performed comparative perilymph permeability determinations of the three cephalosporins that we deemed to have the most clinical potential in these varied situations. Perilymph pharmacokinetic profiles were established for ceftazidime, cefuroxime, and cefotaxime and its metabolite desacetylcefotaxime in 36 guinea pigs by using the technique of high-performance liquid chromatography. At 1, 2, 3, 4, and 6 hours after intravenous administration of the three cephalosporins at a dose of 100 mg/kg of body weight, ceftazidime consistently exhibited the highest perilymph concentration. Desacetylcefotaxime showed the next highest capacity for penetration into perilymph. Keeping in mind that the choice of drug for the treatment of suppurative labyrinthitis should be based foremost on culture and sensitivity studies, we consider ceftazidime to be the first-line agent for treatment and prevention of both meningogenic labyrinthitis and labyrinthitis complicating acute or chronic otitis media.

KEY WORDS — cephalosporins, labyrinthitis, meningitis.

INTRODUCTION

Suppurative labyrinthitis is an uncommon but devastating problem encountered by the otolaryngologist. It is characterized by a permanent profound sensorineural hearing loss and complete unilateral loss of vestibular function. It can originate from a meningeal infectious process (meningogenic), chronic or acute otitis media (otogenic), or, rarely, a distant infectious process (hematogenic). In meningogenic labyrinthitis, pus and bacteria invade both labyrinths, producing well-defined pathologic changes. It may result in complete bilateral deafness and loss of vestibular function, the most devastating form of this disorder. In industrialized countries, meningococcic labyrinthitis appears to be one of the most common causes of profound childhood deafness beyond the neonatal period. It is believed that meningococcic meningitis is responsible for up to one third of the cases of acquired profound deafness in children.[1-3]

The treatment of suppurative labyrinthitis is aimed at prompt recognition and early antibiotic therapy to save any degree of residual hearing. Perhaps just as important is the use of prophylactic antibiotics under circumstances predisposing the labyrinth to infection. These are standard clinical principles in otolaryngology, yet the most appropriate antibiotic for these clinical situations remains unknown. The choice of drug should be based foremost on culture and sensitivity studies, but given an organism susceptible to several antibiotics, which one should be used? The importance of this question arises from the existence of a blood–cerebrospinal fluid (CSF)–perilymph (PL) barrier.[4]

Cephalosporins are nonototoxic antibiotics that provide coverage for almost all bacteria that can cause suppurative labyrinthitis. We have previously reported individual pharmacokinetic profiles of the cephalosporin antibiotics ceftazidime (CTD) and cefuroxime (CRX) within PL, CSF, and plasma.[5,6] The present work was designed to compare their PL permeability with that of cefotaxime (CTX) and its metabolite desacetylcefotaxime (dCTX), and further evaluate the possibility of using these cephalosporins for the treatment of suppurative labyrinthitis. We studied CSF profiles concurrently to enhance our knowledge of the overall relationship between the two fluid compartments, and to find the drug with appropriate capacity to penetrate both PL and CSF so that bacterial meningitis and labyrinthitis may be treated simultaneously.

MATERIALS AND METHODS

Thirty-six albino guinea pigs of either sex, weigh-

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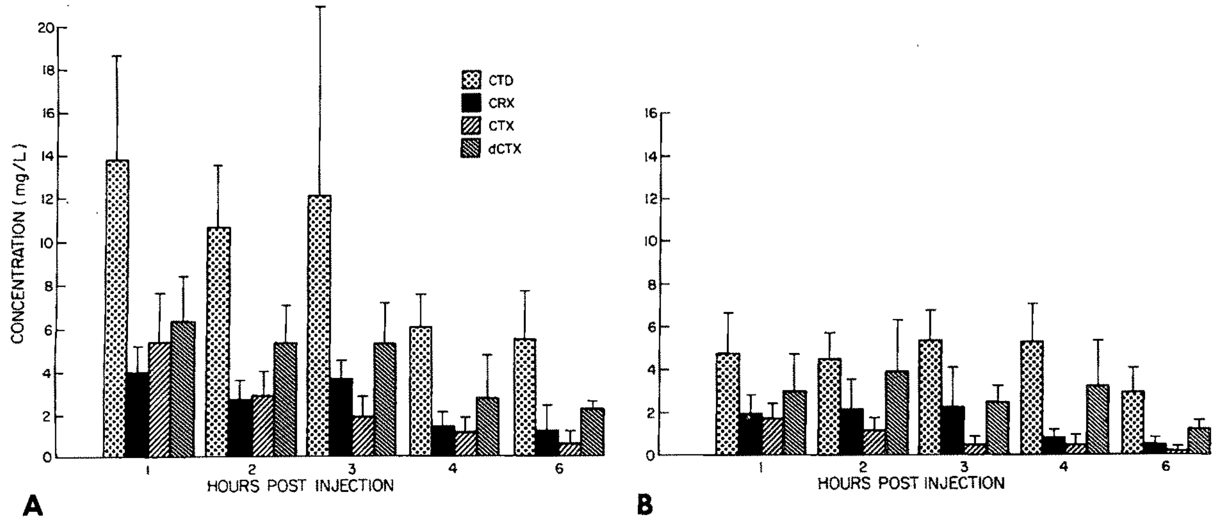

Mean comparative concentrations (±1 SD) of ceftazidime (CTD), cefuroxime (CRX), and cefotaxime (CTX) and its metabolite desacetylcefotaxime (dCTX) at sequential intervals following single intravenous dose of 100 mg/kg of each drug. A) In perilymphatic fluid. B) In cerebrospinal fluid.

ing 500 to 800 g, with normal Preyer's reflexes, were randomly divided into three drug administration groups, with 12 animals in each group. The animals in the three groups received a single intravenous dose (through an external jugular vein) of CTD, CRX, and CTX, respectively. The dosage of each drug was 100 mg/kg, similar to the recommended dose for several bacterial infections in humans. The number of obtainable PL and CSF samples in each animal was limited by small fluid volumes and the potential for blood contamination with repeated sampling. Therefore, each drug group was divided into two sampling time groups. Half the animals were sampled 1, 2, and 4 hours after administration of these drugs, and the other half were sampled 1, 3, and 6 hours postinjection.

Ten-microliter samples of PL and CSF were collected at each sampling time through the round window membrane and lateral ventricle, respectively. Blood contamination of the specimens, detectable by visible discoloration of the fluid, did not occur with the sampling techniques used, which we have previously reported in detail.[5] Each sample was placed in a conical glass micro-insert vial (200 μL) and immediately stored at –60°C until analysis.

The analyses of CTD, CRX, CTX, and dCTX concentrations in both PL and CSF were performed on a Hewlett Packard (HP) 1090 high-performance liquid chromatography (HPLC) system with an HP-1050 variable wavelength detector set at 254 nm and an HP-3393 computing integrator. The column was 10 × 0.32 cm inside diameter, packed with Spherisorb-C8 (5 μm). It was maintained at 40°C throughout the experiment. The mobile phases were 15% methanol-water for CTD, 20% methanol-water for CTX and dCTX, and 25% methanol-water for CRX. Each was mixed with 0.25% dibutylamine and adjusted to a final pH of 5.0 with orthophosphoric acid. All solvents were of HPLC grade. The injected volume was 10 μL for each sample. The flow rate was maintained at 0.5 mL/min. Under these conditions, the retention times for CTX, dCTX, CTD, and CRX were 8.6, 3.1, 6.0, and 5.0 minutes, respectively.

All CTX, CTD, and CRX assay standards were prepared by dissolving each of the drugs in distilled water and diluting to a concentration of 2 μg/mL. The dCTX (Roussel Uclaf Labs, France) assay standard was prepared by dissolving the powdered drug in distilled water and diluting to a concentration of 2 μg/mL. All PL and CSF samples were thawed at room temperature and directly diluted in the micro-insert vials with 40 μL of the appropriate mobile phase. Injections were made directly from these vials.

RESULTS

The Figure, A, shows the concentrations of the three cephalosporins in PL as a function of time at 1, 2, 3, 4, and 6 hours after intravenous administration of 100 mg/kg of body weight. The different drugs yielded different pharmacokinetic profiles. Of note, CTD exhibited the highest concentration at all sampling times. Desacetylcefotaxime showed the next highest capacity for penetration into PL. Cefuroxime and CTX were somewhat similar in their permeability.

The Figure, B, summarizes the pharmacokinetic concentration-time profiles of the three drugs in CSF. All show strikingly different individual pharmacokinetic profiles in CSF from those in PL. However, CTD again had the consistently highest concentration in CSF, followed by dCTX, CRX, and CTX.

DISCUSSION

One prerequisite for the successful treatment or prevention of suppurative labyrinthitis with antibiotics is that biologically active concentrations must be reached at the foci of infection. Besides drug pharmacokinetics, many other factors may influence the rate of bacterial killing at the site of the infection, eg, bacterial density, oxygen tension, variation in pH, interactions between microorganisms, and host defense mechanisms. Furthermore, acute inflammation will likely alter the blood-PL barrier. However, a discussion of these issues is outside the scope of this paper, which focuses on the relationship between the comparative PL permeability and the clinical choice of a specific cephalosporin in the treatment or prevention of bacterial labyrinthitis.

Generally, it is believed that only free, unbound antimicrobial agents are able to interact with microorganisms to exert a pharmacologic effect. The cephalosporins used in this study differ significantly in their degree of serum protein binding. In humans, protein binding is 5% for CTD, 33% to 50% for CRX, and 55% for CTX.[7-9] These differences allow for evaluation of the relationship between protein binding and the ability of cephalosporins to penetrate PL. There is some evidence in the present study that the higher the degree of protein binding, the poorer the penetration into PL. Ceftazidime, with the lowest serum protein binding, produces the highest penetration into PL. Of interest, CTD has an initial rapid increase in PL concentration, with a prolonged stable duration. In CSF there is a slower and lower increase in concentration. On the other hand, CRX and CTX, with similar higher serum protein binding, produce lower concentrations in PL and have similar pharmacokinetic profiles in PL and CSF. It is therefore important to recognize that the degree of protein binding may be used to predict PL permeability of cephalosporins.

Another primary concern of the clinical otolaryngologist is to know whether or not the cephalosporins reach high enough concentrations in PL to exceed the minimal inhibitory concentrations (MICs) of the major pathogens that can cause suppurative labyrinthitis.[10-16] *Neisseria meningitidis, Hemophilus influenzae*, and *Streptococcus pneumoniae* are the three major pathogens of suppurative labyrinthitis that may result from bacterial meningitis, and the latter two may also cause acute otitis media. The MIC values against both ampicillin-susceptible and ampicillin-resistant *H influenzae* strains are 0.25 to 0.5 mg/L for CRX, 0.08 to 1 mg/L for CTX, 0.12 to 0.25 mg/L for dCTX, and 0.015 to 0.12 mg/L for CTD. Against *N meningitidis* strains, the MICs are 0.06 to 0.25 mg/L for CRX, 0.008 mg/L for CTX, 0.008 mg/L for dCTX, and 0.008 to 0.03 mg/L for CTD. For penicillin-resistant *S pneumoniae*, the MICs are 0.02 to 0.16 mg/L for CRX, 0.04 to 1.0 mg/L for CTX, 0.06 to 0.25 mg/L for dCTX, and 0.03 to 0.25 for CTD. In our study, 6 hours following intravenous administration, the mean PL concentrations of all three cephalosporins and the dCTX metabolite of CTX remained higher than the reported MICs of these drugs for meningococci, pneumococci, and *H influenzae*.

On the basis of the pharmacokinetics of the three cephalosporins summarized in this report, CTD should be the most effective agent for the treatment of bacterial labyrinthitis caused by the aforementioned microorganisms. Fortunately, CTD, unlike CRX and CTX, is also highly active against *Pseudomonas aeruginosa*, the major pathogen of suppurative labyrinthitis complicating chronic otitis media. Since dCTX 1) has antibacterial activity similar to that of CRX, 2) can achieve a higher concentration than CRX in both PL and CSF, and 3) is synergistic with its parent drug for the three main meningeal pathogens, CTX may also be recommended for patients with suppurative labyrinthitis.

Results from clinical studies prove that cephalosporins penetrate inflamed meninges much better than noninflamed meninges.[17,18] It seems likely that similar trends might be found in the penetration of CTD, CTX, CRX, and dCTX into PL in patients with suppurative labyrinthitis. Thus, the more than adequate penetration of all cephalosporins through the normal blood-PL barrier shown by our results should only be enhanced by the presence of acute inflammation. Our predicted results could be further evaluated by means of the meningogenic suppurative labyrinthitis animal model described by Bhatt et al.[19]

In conclusion, because of its high perilymph permeability and broad coverage of the significant microorganisms, we consider ceftazidime to be the first-line antibiotic for the treatment of both meningogenic and otogenic labyrinthitis. We also recommend ceftazidime as the prophylactic antibiotic of choice in circumstances predisposing the labyrinth to infection. The most likely offending organism would be *P aeruginosa*. Representative clinical examples include surgery for cholesteatoma with lateral canal fistula or invasive inner ear surgery (eg, posterior semicircular canal occlusion, cochlear implantation) in ears predisposed to recurrent otitis media. Cefotaxime remains a reasonable second choice for susceptible organisms.

ACKNOWLEDGMENTS — We thank Pauline Reilly and Victor Batorowicz for their assistance in this study.

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CRANIOFACIAL/CLEFT LIP AND PALATE SOCIETY OF GREAT BRITAIN

The next meeting of the Craniofacial/Cleft Lip and Palate Society of Great Britain will be held April 24-26, 1996, in Eghan, Surrey, England. For further information, contact Dr Debbie Sell, Speech and Language Therapy Department, The Hospital for Sick Children, Great Ormond Street, London, WC1N 3JH, United Kingdom; telephone (UK) -71 405 9200 ext. 5043.

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ACUTE OTITIS MEDIA AND FACIAL PARALYSIS IN CHILDREN

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We reviewed 10 children who presented with facial paralysis after the onset of acute otitis media. The objective of the study was to examine the outcome of facial paralysis in children with acute otitis media treated without facial nerve decompression. Two groups were identified: 8 patients with incomplete paralysis and 2 with complete paralysis. Seven of the 8 patients with incomplete paralysis had rapid return of function after myringotomy and intravenous antibiotics. The eighth patient had delayed recovery requiring 9 months before complete return of function. The 2 patients with complete paralysis required mastoidectomy to control otorrhea and fever after initial myringotomy and antibiotics. Both patients had a prolonged recovery requiring 3 and 7 months for complete recovery. Patients with incomplete paralysis generally show rapid improvement following wide myringotomy and antibiotic treatment. A more protracted recovery may be expected in patients with complete paralysis; excellent return of function is expected when mastoidectomy without facial nerve decompression is employed.

KEY WORDS — acute otitis media, children, facial paralysis.

Incomplete facial paralysis from acute otitis media usually settles after myringotomy and adequate antibiotic therapy. A less-recognized but well-reported entity is the progression to complete paralysis despite seemingly adequate intervention. A wide variety of treatment approaches have been advocated in the literature. These reports have generally dealt with single cases or small groups of rather dissimilar patients, and despite differences in management, all authors report good to excellent recovery in their series. The use of antibiotics alone or with myringotomy is universally accepted as initial management. Controversy arises when considering patients who manifest progression to complete facial paralysis despite these conservative measures. The central issue appears to be the need for exploration and decompression of the facial nerve. The purpose of our study is to examine the outcome of facial paralysis associated with acute otitis media in patients treated without facial nerve decompression.

SUBJECTS AND METHODS

The charts of all patients with acute otitis media and facial paralysis treated at Children's National Medical Center over the 10-year period from 1983 to 1992 inclusive were reviewed. Excluded from study were patients with previously known facial paralysis, facial paralysis of traumatic or iatrogenic origin, and patients with facial paralysis from unusual infectious causes such as Ramsay Hunt syndrome, which did not conform to the classic pattern of acute suppurative otitis media. Parameters that were identified were age, gender, duration of otitis media and facial paralysis prior to presentation, results of electromyography and nerve conduction studies, radiographic findings of computed tomography (CT) scanning of temporal bones, audiologic findings, surgical treatment, and outcome.

RESULTS

Ten patients met the criteria for study. Two main groups were identified, consisting of 8 patients with incomplete paralysis and 2 patients with complete paralysis.

The mean age of patients with incomplete paralysis was 5.5 years, with a range of 10 months to 15 years. There were 6 boys and 2 girls. The mean duration of infection prior to presentation was 3.5 days, with a mean duration of facial paralysis of 1.2 days. Seven patients underwent CT scanning of the temporal bones; it showed opacification of the middle ear and mastoid air cell system without evidence of bony erosion in all cases. Audiologic assessments were performed in 3 patients, indicating mild to moderate conductive hearing loss in 2 patients and normal hearing thresholds in the third patient. Middle ear effusion cultures were negative in 7 patients secondary to previous antibiotic intake, and one culture grew *Staphylococcus epidermidis*. All patients received broad-spectrum intravenous antibiotics (chloramphenicol and penicillins in the early 1980s, and cefuroxime

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FINDINGS AND CLINICAL COURSE OF PATIENTS

Patient No.	*Age*	*Duration of Paralysis (d)*	*Degree of Paralysis*	*CT Scan Findings*	*Audiologic Findings*	*Nerve Conduction Studies*	*Surgery*	*Duration of Recovery*
1	11 y	6	Incomplete (transient complete)	Opacification, coalescence	Normal	Negative MST at 10 d but facial movement at 11 d	Myringotomy	Normal after 9 mo
2	3 y 4 mo	1	Incomplete	NP	Mild to moderate conductive hearing loss	NP	Myringotomy	60 d
3	16 mo	1	Incomplete	Opacification, coalescence	NP	NP	Myringotomy and tympanostomy tube	10 d
4	14 y 11 mo	1	Incomplete	Opacification, coalescence	40 dB hearing level	NP	Myringotomy	14 d
5	4 y 9 mo	1	Incomplete	Opacification, coalescence	NP	NP	Myringotomy	30 d
6	3 y 11 mo	5	Incomplete	Opacification, coalescence	NP	NP	Myringotomy	1 d
7	1 y	1	Incomplete	NP	NP	NP	Myringotomy and tympanostomy tube	21 d
8	1 y 8 mo	4	Complete	Opacification, coalescence	30 dB hearing level in sound field	<10% at 7 d; no response at 10 d	Myringotomy and tympanostomy tube; mastoidectomy	90 d
9	10 mo	1	Incomplete	Opacification, coalescence	NP	NP	Myringotomy	11 d
10	7 y	1	Complete	Opacification, coalescence	Left 65 dB hearing level conductive hearing loss	No response at 7 d	Myringotomy and tympanostomy tube; mastoidectomy	7.5 mo

MST — maximum stimulation test, NP — not performed.

for the past 2 years) and myringotomy and drainage with or without tympanostomy tube. Nerve conduction studies were not performed in any patient with incomplete paralysis. The period for recovery ranged from 24 hours to 9 months, with a mean of 7.4 weeks. Excluding 1 patient (patient 1) with prolonged recovery, the mean duration of recovery was 21 days.

Both patients with complete paralysis were male, 20 months and 7 years of age, with a mean age of 4.3 years. The duration of infection prior to presentation was 5 days and 3 weeks, respectively, with a mean of 13 days, and the duration of facial paralysis was 5 days and 1 day, respectively, with a mean of 3 days. Both patients underwent an intact canal wall mastoidectomy without decompression of the facial nerve after fever and otorrhea continued for longer than 1 week despite intravenous antibiotics and myringotomy with drainage. Both patients attained complete recovery of facial function that required 3 months (patient 8) and $7^{1}/_{2}$ months (patient 10). The findings and clinical courses of all patients are summarized in the Table. Patients 8 and 10 are described in detail below.

Patient 8. This 20-month-old boy presented with a 5-day history of bilateral otitis media and decreased right facial movements despite 4 days of broad-spectrum oral antibiotic therapy (Pediazole). A red, bulging, opaque right tympanic membrane was noted, with complete right facial paralysis present. Audiologic assessment showed a 30-dB hearing level by sound field behavioral assessment. The patient was initially treated with broad-spectrum intravenous antibiotic therapy (Timentin; ticarcillin disodium–clavulanate potassium) and wide myringotomy with drainage (cultures showed no growth). Seven days following the onset of paralysis there was a 90% reduction in facial function by nerve conduction studies, and no activity was noted on repeat testing at 10 days. The otorrhea and fever had continued despite antibiotic therapy and local care.

An intact canal wall mastoidectomy was performed, at which time granulation tissue was noted throughout the middle ear and mastoid, but particularly over the ponticulus and the horizontal portion of the facial nerve. No dehiscence of the facial nerve was noted. Biopsies of the granulation tissue were consistent with bacterial infection, and the facial nerve was not decompressed.

Postoperatively, the otorrhea rapidly settled, and the patient was discharged after completing a 7-day course of intravenous antibiotic therapy. Facial nerve function returned to normal after 90 days, and otologic and audiologic (evoked response) assessments showed normal results.

Patient 10. This 7-year-old boy presented with a 3-week history of left otitis media with purulent otorrhea despite oral antibiotic intake (cefaclor 10 days, followed by 10 days of Septra). The patient was admitted to the hospital when left facial weakness had been noted for 24 hours. A left tympanic membrane perforation and purulent otorrhea were present, and a marked decrease in facial movement was noted in all branches. Intravenous antibiotic therapy was initiated (cefuroxime and oxacillin sodium, which were changed to ceftazidime and clindamycin after cultures of ear drainage grew *Pseudomonas aeruginosa*) and drainage was enhanced by wide myringotomy. Computed tomography scanning of the temporal bones revealed opacification of the mastoid air cell system without evidence of coalescence or bony erosion. Audiologic assessment revealed a maximum conductive hearing loss on the left with a pure tone average of 60 dB hearing level. Facial weakness progressed to complete paralysis over a 4-day period. Electromyography and nerve conduction studies confirmed no evidence of facial nerve function. A left intact canal wall mastoidectomy was performed to control continued otorrhea with fever. Extensive granulation tissue was noted, particularly over the horizontal portion of the facial nerve, without evidence of dehiscence of the bony canal. Biopsies were consistent with bacterial infection, and the facial nerve was not decompressed. The otorrhea and fever rapidly settled. A 3-week course of home intravenous antibiotics was given. Complete facial nerve function returned after 7½ months, except for mild asymmetry noted at the left oral commissure during forced smiling, with no evidence of synkinesis present. Otologic and audiologic assessment results were normal.

DISCUSSION

The incidence of facial paralysis associated with acute otitis media has decreased with the advent of combined antibiotic therapy and myringotomy.[1] Because of the infrequent presentation of this condition, few otolaryngologists have had a broad experience with a large number of cases.

The exact pathophysiology is not known. The potential processes that have been proposed have been 1) direct involvement of the facial nerve by the infectious process and surrounding osteitis accompanied by swelling of the facial nerve within the confines of the fallopian canal, 2) demyelination of the facial nerve secondary to the presence of bacterial toxins, and 3) ischemia of the facial nerve secondary to thrombosis of the vasa nervorum, leading to infarction. Involvement of the facial nerve is felt to be secondary to direct contact with the inflammatory process within the middle ear and mastoid through natural dehiscences of the fallopian canal or direct involvement secondary to the osteitic process.[2] Despite the presence of dehiscences of the fallopian canal in more than 55% of temporal bones studied,[3] the rarity of facial paralysis with acute otitis media would suggest that the perineurium is quite resistant to involvement by the infectious process.

Ballance and Duel[4] noted osteitis of the bone surrounding the fallopian canal and advocated aggressive removal with facial nerve decompression to control what appeared to be direct invasion of the nerve. Kettle[5] did not encounter osteitis in the majority of patients in his experience of 18 cases; he proposed the concept that bacterial toxins are responsible for the nerve dysfunction and advocated aggressive control of the infectious process to be the primary indication for surgical therapy rather than the presence of facial paralysis itself. To date, however, bacteriologic studies have failed to identify any specific bacterial neurotoxins. Histopathologic studies have led Antoni-Candela and Stewart[6] and Graham[7] to propose that thrombosis of the vasa nervorum leads to ischemia and death of the nerve. Although the exact pathophysiology is not known, the universal finding at the time of decompression is the presence of marked swelling of the facial nerve at the point at which the inflammatory process is greatest, usually at the horizontal portion of the nerve and then extending along the distal segment.[4,8,9]

The largest series of cases of facial paralysis with acute otitis media emanated from the preantibiotic era, when the condition was a matter of great interest for the pioneers of facial nerve surgery.[5] In the postantibiotic era, few authors report a large experience with this condition. While there is general agreement that initial management should consist of myringotomy and intravenous antibiotic therapy, no consensus has emerged regarding management of complete paralysis.[7-15] Proponents of aggressive management advocate decompression of the nerve when complete paralysis exists in the presence of one or more poor prognostic indicators (decreased tearing, decreased salivary flow, abnormal results on nerve conduction studies).[4,8,9,11] In Hof's[8] series of 7 cases, 2 out of 3 patients presenting with complete paralysis within 6 days of onset of weakness underwent mastoidectomy and decompression of the facial nerve, with excellent recovery noted in both cases. Surgery was deferred on the third patient because of a response to myringotomy and antibiotic treatment. May[9] refers to his experience with 19 children who underwent surgical exploration for complete paralysis. Ten patients were found to have mastoiditis, and all underwent facial nerve decompression. Twelve of the 19 patients had complete recovery, 6 had fair, and 1 had poor return of function. In this brief account, the author does not state which patient in each group underwent decompression of the nerve.[9] Shott and Myer,[11] in a letter to the editor, reaffirmed the position of Hof and May, but did not provide details of their own series of patients.

Others favor a more conservative approach, advocating mastoidectomy alone in the acute setting, with facial nerve decompression reserved for patients with prolonged recovery.[5,7,10-13,15] The series of 4 cases reported by Kamitsuka et al[10] included 1 patient with complete facial paralysis and no response to electrical stimulation who underwent simple mastoidectomy without facial nerve decompression. Complete return of function was noted 1 month following surgery. Jacobsson et al,[15] in their report of 2 cases, observe excellent return of function with myringotomy and antibiotic therapy alone. They supported the use of conservative measures, preferring mastoidectomy alone without decompression for patients in whom rapid recovery is not observed.

In the absence of frank invasion and disruption of the nerve, vascular thrombosis with infarction is the most likely cause, and in this situation, the neural tubes would be expected to be relatively intact. Unlike circumstances in which physical impingement at a specific site produces compression of the nerve and interferes with reinnervation, the value of surgical release in this situation is somewhat in doubt. In contrast, aggressive removal of granulation tissue and extensive dissection may expose an edematous and potentially ischemic nerve to further injury. Despite wide variations in therapeutic approaches, all authors report good to excellent recovery of facial function when more invasive measures such as mastoidectomy are employed to rapidly settle the continuing infectious process, whether or not the facial nerve is decompressed. This is likely a testament to the regenerative ability of the nerve in the presence of a significant injury, rather than to the superiority of one treatment form over another. The goal of therapy, therefore, should be to use the most conservative approach that effectively deals with the inflammation around the nerve, thereby avoiding further injury.

In our series we identified two main groups of patients. Patients with incomplete paralysis generally improve rapidly following myringotomy and antibiotic therapy. The patient with prolonged recovery in this group (patient 1) showed return of facial function within 24 hours. Even though complete paralysis was transiently observed, the subsequent improvement would mean that this patient would not satisfy the critera for facial nerve decompression proposed by other authors.[9,11] Nonetheless, a protracted recovery may be observed in patients who sustain significant facial injury, even when complete paralysis does not occur. It is therefore interesting to observe that the period of time for complete recovery was longer than that observed for either patient with complete paralysis.

The second group consisted of patients who progressed to complete paralysis in the setting of continued fever and otorrhea. The presence of an ongoing infectious process manifest by otorrhea and fever, despite conservative measures, provided conditions for progression of the facial nerve injury. With the combination of complete loss of function and abnormal nerve conduction, both patients would have met the proposed criteria for facial nerve decompression.[9,11] Excellent recovery, however, was observed in each case after simple mastoidectomy alone. We treat our patients with initial myringotomy and broad-spectrum intravenous antibiotics until improvement in facial function is noted. Mastoidectomy is reserved for progression of facial paralysis in the setting of continued otorrhea and fever and to rule other unusual causes such as fungal disease, tuberculosis, neoplasms, eosinophilic granuloma, or autoimmune disorders that may masquerade as acute otitis media with mastoiditis.[15,16]

We have found that intensive measures to control the infectious process without decompression have provided excellent return of facial function, and find no significant difference in outcome compared to the experience of others reporting in the literature. Although the number of patients in our series is small, to the best of our knowledge, our experience represents the largest case-controlled study of this problem since Kettle's[5] report of 18 patients in 1943.

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CLINICAL EVALUATION OF EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY FOR SALIVARY STONES

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The treatment of sialolithiasis is discussed in this report. Generally, stones within the distal salivary duct are easily removed by transoral ductotomy, although proximal stones are usually treated by excision of the salivary gland and its duct. Since 1980, extracorporeal shock wave lithotripsy (ESWL) has been in clinical use for the treatment of renal and gallbladder stones. We used this technique as a treatment for sialolithiasis. We undertook ESWL on 14 submandibular gland stone patients, 1 parotid gland stone patient, and 3 submandibular duct stone patients. In 10 of 14 submandibular gland stone patients and 1 parotid gland stone patient, clinical symptoms such as pain and swelling disappeared without excision of the affected salivary gland. Stones larger than 10 mm seem to have a tendency to form *Steinstrassen*. Although computed tomography findings correlate with success in breaking up gallstones, they did not predict success for salivary stones. We conclude that sialolithiasis is treated successfully without adverse effects by ESWL in selected patients.

KEY WORDS — computed tomography, extracorporeal shock wave lithotripsy, *Steinstrasse*.

INTRODUCTION

For the treatment of renal stones, extracorporeal shock wave lithotripsy (ESWL) has been proven to be effective since 1980.[1] Since 1986, this technique has been applied to gallstones and has become well established in gastroenterology.[2] As for salivary stones, it was not a clinically useful method. The focus of the shock wave was so large that central nervous system structures were affected; therefore, the shock wave–producing systems, such as the spark gap method and the electromagnetic method, were not available for the head and neck area. Recently, the piezoelectric shock wave lithotripter, the focus area of which was very small, was developed. In 1989, Iro et al[3] reported the first successful case treated with this ESWL system. In 1992, they reported successful results in 19 salivary stone patients treated by ESWL.[4] We have treated 18 salivary stone patients by ESWL and evaluated whether the factors that predict the benefit of ESWL for renal stones and gallstones are applicable to salivary stones. In this report, we introduce our treatment of sialolithiasis and show our results.

MATERIALS AND METHODS

Patients. The following criteria were required for patients with sialolithiasis to receive ESWL treatment: 1) a history of salivary gland swelling or pain attributable to salivary stones, 2) salivary stones visible on a 3.5-MHz monitor ultrasound B-mode scanner, 3) the patient's agreement to ESWL treatment, and 4) a functional salivary gland as verified by the chewing gum test. After chewing sour-tasting gum, those who showed swelling or pain of the affected gland were considered gum test–positive. If the salivary gland is nonfunctional, ESWL is not used, because productivity of saliva is essential for excretion of the salivary stones via the duct. The age of the patient, the site of the stones, the diameter of stones, and the number of stones were not considered limitations.

Computed Tomography. Computed tomography (CT) scans of 12 stones in 11 patients were obtained as contiguous 5-mm sections. The CT value of each stone was measured by an electronic cursor that uses a computer program to analyze a certain region of interest on the CT scans. A circular region of interest was placed at 1 or 2 mm in diameter. Then, inside each stone's boundary, the average CT value of each stone was determined. All scans were performed on a Pro Seed (Yokogawa Medical Systems, Japan) CT scanner.

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CLINICAL DATA OF EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY

Patient No.	*Age*	*Sex*	*Gland*	*No. of Stones*	*Diameter (mm)*	*No. of Shock Wave Exposures*	*CT Value*	*Outcome*
Submandibular gland								
1	30	F	R	2	4.5	2	450, 480	1
2	34	F	L	1	6	1	600	2
3	79	M	R	1	5	1	ND	2
4	37	M	R	1	11	7	806	1
5	23	F	R	1	7	1	ND	1
6	39	M	R	1	20	3	1,320	0
7	55	M	R	2	14.9	3	906*	0
8	32	M	L	1	8	3	ND	1
9	40	M	R	1	18	1	ND	0
10	17	F	L	1	5	1	ND	1
11	61	M	R	2	9.5	1	1,191*	0
12	46	M	R	1	7	5	859	1
13	44	F	R	1	9	3	1,226	1
14	56	F	R	1	10	2	1,113	1
Wharton's duct								
15	26	M	R	1	11	1	806	1
16	59	M	L	1	12	1	ND	1
17	30	F	L	1	8	1	ND	1
Parotid gland								
18	43	F	R	1	15	3	1,755	2

ND — not determined. For outcome, 0 — gland resection, 1 — ductotomy or watching, 2 — excreted via duct.
*Value of larger stone.

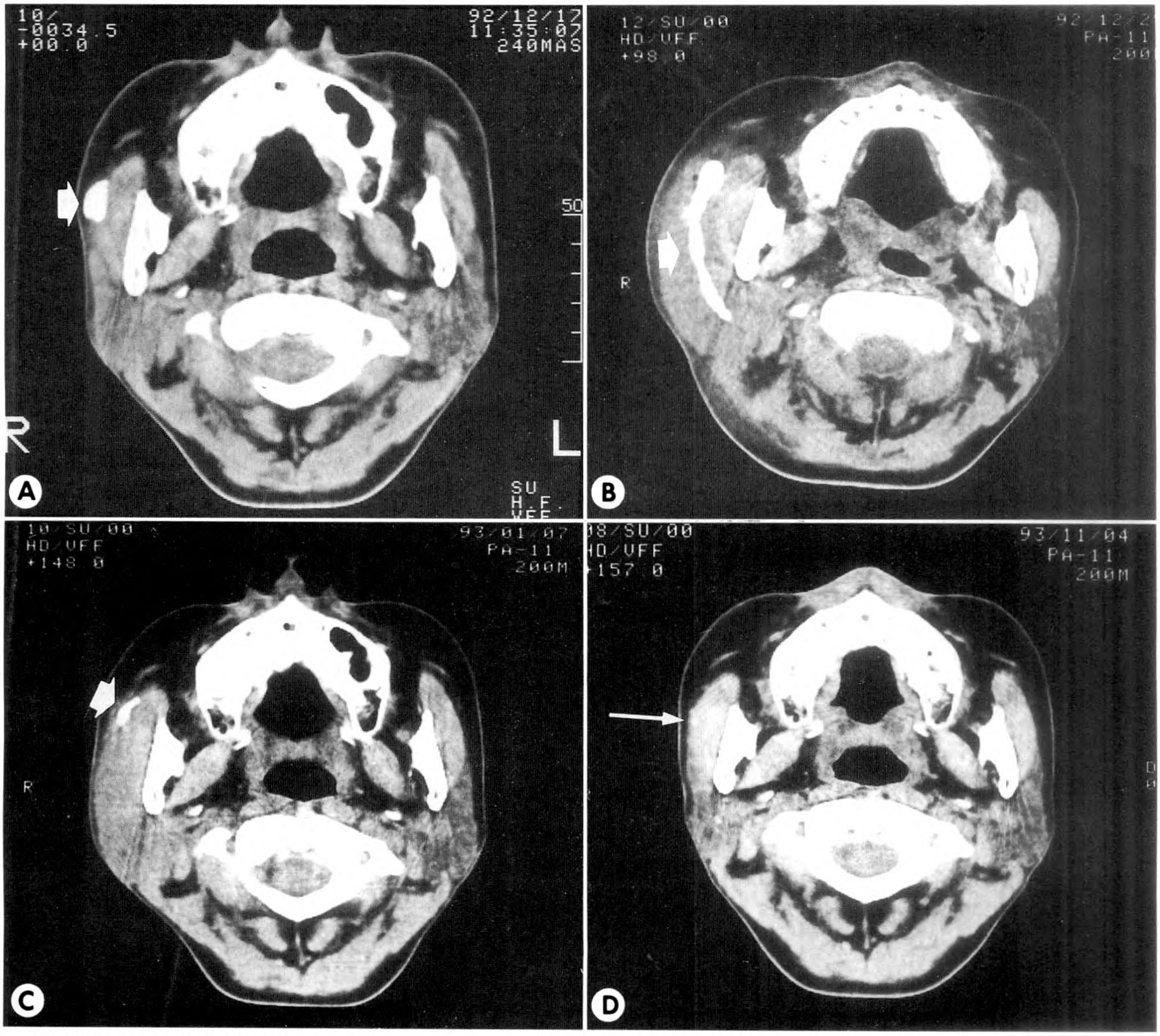
Equipment and Shock Wave Treatment. We used the piezoelectric lithotripter LT-01 (EDAP, France) for generating shock waves. The location of the stones was determined in two planes by means of an integrated, real-time ultrasound B-mode scanner. Then, the pathways of the shock waves were examined alternatively and the shock wave generator was manipulated three-dimensionally under a sonographic monitor until the aimed-at stone was on the shock wave focus. The pulse frequency of the shock wave could be varied by eight steps ranging from 1.25 to 160 Hz, and the energy of the shock wave on the focus (pressure) could be set from 0 to 900 bar. Iro et al[4] reported that in in vitro fragmentation experiments, adequate fragmentation of surgically removed calculi could be achieved to the same degree at the lowest-intensity setting (400 bar) of their shock wave generator as at the higher-intensity settings. Following their results, we usually treated the patients with a 2.5-Hz, 400-to-600-bar shock wave for 50 minutes. About 7,500 shock waves were used in one session. All patients were given analgesic suppositories before ESWL treatment. None required anesthesia or sedatives.

Examination Before and After Treatment. All patients underwent radiography and ultrasonography evaluation before ESWL treatment. The serum amylase level was also investigated before and after treatment in each case. In 10 of 18 patients, CT evaluation of stones was performed 1 to 24 hours before treatment. Patients were examined on the seventh day after the first treatment, unless complications such as swelling of the treated gland occurred. If there were residual calculi in the gland, the patient chose one of three treatments: transoral ductotomy, excision of the affected salivary gland, or ESWL. Those who chose ESWL were usually treated on that day or the next day. As for fragmented calculi excreted into the duct of the gland, ducts blocked with these calculi were drained by transoral ductotomy, and those not blocked were watched. Patients cured by ESWL treatment were instructed to undergo monthly follow-up, but most of them did not follow our recommendation. We supposed that they remained trouble-free. Patients who received submandibulectomy were not scheduled for follow-up.

Adjuvant Treatment. Massage of the affected salivary gland and sour-tasting foods were recommended. Drugs such as analgesics were not needed immediately after treatment.

RESULTS

Twenty-five patients with salivary gland and duct stones visited our hospital from April 1991 to Febru-



(Patient 18) Morphologic change of salivary stone (arrows) before and after extracorporeal shock wave lithotripsy. A) Before first treatment. Salivary stone is in anterior portion of right parotid gland. B) One day after second treatment. Fragmented stones exist along main duct of parotid gland and form *Steinstrasse.* C) Two weeks after second treatment. Most of fragmented stones were excreted. D) Six months after third treatment. Small calcification exists. This may be residual fragmented stone or recurrent stone.

ary 1994. Of 16 patients with submandibular gland stones, 14 were treated with ESWL (see Table). The other 2 patients preferred excision of the gland. Of 7 patients with submandibular duct stones, 3 patients were treated with ESWL (see Table). The other 4 patients preferred transoral ductotomy. Of 2 patients with parotid gland stones, 1 patient (patient 18) was treated with ESWL. In the other patient, ESWL was contraindicated because the stone was too small to be detected on 3.5-MHz monitor sonography with the LT-01. He was treated with transoral ductotomy, and the stone was excreted via the resected duct (not listed in Table).

Except for the stones of patients 7, 9, and 11, all submandibular gland stones were fragmented or diminished in size and the symptoms disappeared. Patients 7 and 11 were treated with excision of the glands. The duct of patient 9 ruptured to the floor of his mouth 7 days after initial ESWL treatment. In some patients, a "stone street" (*Steinstrasse*), or alignment of fragmented stones, was observed, resulting in a choked duct. Although the stone of patient 6 was almost fragmented, the duct within the gland was choked by the fragments, transductal drainage was not effective, and excision of the gland was required. In patient 14, although the same *Steinstrasse* phenomenon occurred, we were able to use transductal drainage and prevent gland excision. As for submandibular gland stones, 10 of the 14 patients (71.4%) benefited from ESWL treatment.

None of the submandibular duct stones treated with ESWL could be disintegrated into sludge, and transoral ductotomy was performed 7 days after the initial ESWL treatment. One parotid gland stone (patient 18) was totally fragmented after the second ESWL treatment, but the diameter of the stone was so large that the parotid gland duct was also blocked by the fragments, as in patients 6 and 14. The fragments were gradually excreted via the duct, and transoral ductotomy was not necessary, although the affected gland was swollen by salivary stasis for a few days (see Figure).

The CT values were examined on 12 stones. The average CT value was 960 Hounsfield units. There was no correlation between the CT values of the stones and the outcome of ESWL treatment.

As a side effect, all except the parotid gland stone patients felt pain in the mandible and around the stone as the pressures on focus were increased more than 500 bar. Small petechial bleeding of the skin was observed in 4 patients; this bleeding had disappeared before the next examination. Transient serum amylase level elevation was observed in 4 patients whose ducts were blocked (patients 1, 6, 14, and 18), although these elevated levels were almost within the normal range for our hospital. Amylase levels of patients 1, 14, and 18 returned to normal after drainage or spontaneous excretion, but in 1 patient (patient 6), amylase elevation remained until surgical removal of the affected submandibular gland. Neither facial nerve palsy nor damage of the teeth was observed in any patient.

DISCUSSION

The treatment of sialolithiasis was by two methods, namely, dissection of the duct and removal of the salivary gland, until the development of the piezoelectric lithotripter. Brouns et al[5] reported, in in vitro research, disintegration of amalgam restorations and serious damage to the enamel of molars as effects of ESWL used for salivary stones. This complication was due to the shock wave–producing instrument. Before the introduction of piezoelectric lithotripsy, all 14 submandibular gland stone patients and 1 parotid gland stone patient (see Table) were candidates for surgical removal of the affected salivary glands. The very small focus area of the piezoelectric lithotripter enables us to treat salivary stone patients without disintegration of amalgam restorations or damage to the enamel of the molars. So, ESWL has become an effective treatment for patients whose salivary stones are found within the gland.

Mueller et al[6] reported that ESWL of impacted ureteral stones was not as successful as that of renal stones. They explained that initial fragmentation of the outer shell of impacted stones absorbed or reflected shock wave energy. Moreover, concretions require a surrounding liquid for good disintegration by focused shock waves. Any other medium except water around the stone (for instance, edematous mucosa and granulation tissue) or extrinsic pressure on the stone keeps fragmented small stones in the same place. If there is no space for the fragmented stones to fall apart, most of the shock wave energy seems to be absorbed within the multiple interfaces of the fragmented outer shell.[6] The stones within intraglandular duct systems are usually surrounded by saliva, although those excreted to Wharton's duct are often surrounded by edematous mucosa. The diameter of the stones visible in 3.5-MHz ultrasonography is greater than the diameter of Wharton's duct, so those stones receive extrinsic pressure from the Wharton's duct wall. This situation resembles that of ureteral stones, which receive extrinsic pressure from the ureter. If a shock wave hits an embedded Wharton's duct stone, the first fragments of outer shell cannot fall away. So, we distinguished intraglandular duct stones from Wharton's duct stones. We could not distinguish stones originating in the parenchyma of the salivary glands from intraglandular duct stones. In fact, ESWL of submandibular duct stone patients was also unsuccessful, although they were treated only once. Transoral ductotomy is not a very invasive method and stones within the duct seem easily removed this way. Hence, ESWL is contraindicated in salivary duct stones.

There is a tendency that as the diameter of stones becomes larger, the effect of ESWL becomes smaller, although the tendency is not statistically significant. Although stone fragmentation was achieved in 12 of 15 salivary gland stone patients, complete disappearance of stones occurred in only 3 of 12 patients. It is one thing to break up calculi; it is another thing for them to be excreted via the duct. Large calculi showed a tendency to choke the duct and form a *Steinstrasse*. Stones larger than 10 mm seem to have a tendency to form *Steinstrassen*. Three of 14 patients had two stones in the submandibular gland. In 2 of the patients, fragmentation of the stones was not observed, and they underwent submandibulectomy. Patients who had two salivary stones required two ESWL treatments, indicating that the effect of ESWL decreases as the number of stones increases. Iro et al[4] used the Piezolith 2300 (Fa. R. Wolf GmbH, Knittlingen, Germany) lithotripter and reported that in 11 of 19 patients with salivary gland stones, all fragments were discharged via the duct without any invasive treatment 4 months after initial lithotripsy and no difference could be found between the stone-free patients and the patients with residual fragments regarding the diameter of the calculi. The difference in results seems to be caused by several factors, namely, the difference in lithotripter, the location of the stones, the variation of the diameter of the calculi, the number of the stones within the affected glands, and the observation period. In particular, Iro et al treated patients with solitary stones, and the sizes of the stones varied between 6.7 and only 12.0 mm. In our cases, 2 of 3 multiple stone patients received gland excision, and 5 of 18 patients had stones larger than 12 mm. So, the size of the stones and the number of stones have a great influence on the result of ESWL.

It is useful, for renal stones and gallstones, to predict the chemical composition of the stone, because the chemical composition influences the fragmentation of the stone.[7] It is generally accepted that CT values correlate with the content volume of calcium. Staritz et al[8] reported that gallstones showing low CT values were easily fragmented compared with those showing high CT values. We investigated the CT values of 12 salivary gland and duct stones before initial ESWL treatment. As for salivary stones, there was no significant correlation between CT values and our results in breaking up the stones.

There were no significant side effects such as facial nerve palsy, lingual nerve palsy, hypoglossal nerve palsy, or disintegration of molar teeth. Although complete disappearance of the stones was observed in 3 of 15 salivary gland stone patients, in 11 of 15 patients disappearance of symptoms was obtained. We cannot discuss the rate of complete disappearance of residual fragmented stones or of stone recurrence.

CONCLUSION

Extracorporeal piezoelectric shock wave lithotripsy is effective for salivary gland stones. The effect of ESWL decreases as the diameter and the number of the stones increase. Salivary duct stones are not amenable to ESWL. They should be treated with transoral ductotomy. If large stones are fragmented, they can be expected to form a *Steinstrasse* and might require transoral ductotomy or surgical removal of the affected gland. The CT values of salivary stones do not predict the effect of ESWL, although those of renal stones and gallstones do. No significant side effects were observed during or after ESWL treatment.

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EPISTAXIS BALLOON CATHETER STABILIZATION OF ZYGOMATIC ARCH FRACTURES

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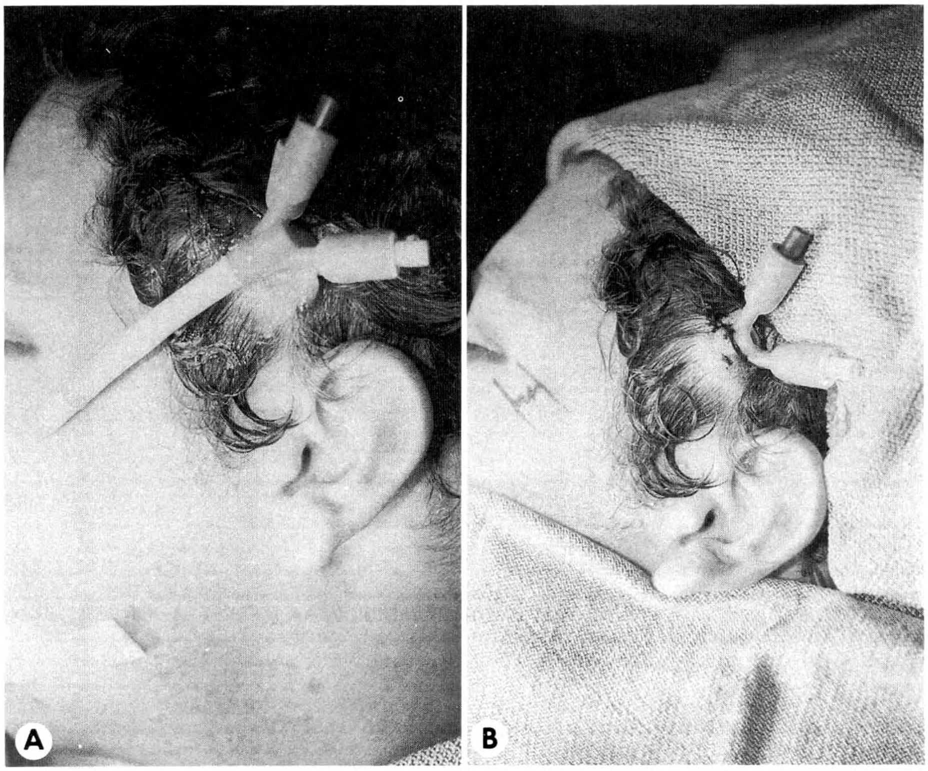
Isolated zygomatic arch fractures represent about 10% of facial fractures. Most are easily reduced, but about 7% require fixation. Stabilization with plates, transcutaneous wiring, and various packing materials may involve additional morbidity and other drawbacks. A double balloon epistaxis catheter may be inserted beneath the arch fracture site and inflated for 5 to 7 days as a relatively simple, reliable alternative that involves minimal morbidity.

KEY WORDS — facial fracture, facial trauma, zygomatic fracture, zygomatic fracture repair.

INRODUCTION

Isolated zygomatic arch fractures represent about 10% of facial fractures[1,2] and are usually due to a direct blow from an assault or fight. Symptoms include facial flattening (57%), trismus (45%), subconjunctival ecchymoses (29%), epistaxis (21%), and infraorbital anesthesia (18%); of these fractures, 18% are asymptomatic.[2] Cosmetic deformity and impaired mandibular motion due to coronoid process impingement are the main indications for fracture reduction. Methods include intraoral (Keen) and temporal (Gillies) approaches as well as transcutaneous traction with use of a hook or towel clips.[3] Fixation is not usually required following reduction (only 7.3% in one series of 166 patients[2]), as the temporalis fascia and masseter muscle generally splint the fragments well. We present a simple method of treatment of unstable depressed zygomatic arch fractures that we have used successfully in 6 patients over the past 3 years.



Epistaxis catheter. A) Laid over incision and fracture to gauge depth of insertion. B) Inserted in Gillies incision, inflated, and secured.

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APPROACH

After induction of general anesthesia and using a standard sterile technique, we reduce the fracture by the temporal approach. If the arch is unstable, a double balloon nasal epistaxis catheter (Epistat, Xomed-Treace, Jacksonville, Fla) is used to support the arch. The uninflated catheter is laid on the skin from the incision to 2 to 3 cm below the fracture site to estimate the length of insertion (see Figure, A). The catheter is placed through the Gillies incision to lie medial to the fracture under the temporalis fascia. Both lumens are inflated with sterile saline to provide appropriate support (5 to 7 mL in the proximal [cephalad] lumen and 10 to 15 mL in the distal [caudad] lumen). A submental vertex "bucket handle" radiograph may be obtained to demonstrate reduction. A 2-0 silk suture secures the catheter to the skin above the incision, and a 3-0 nylon purse-string suture loosely closes the incision. Bacitracin ointment is applied to the wound (see Figure, B).

A foam-padded aluminum finger splint is molded and shaped to the face to protect the fracture. The wound is cleaned with peroxide twice a day, and bacitracin ointment is applied three times a day. Dicloxacillin sodium or cephalexin monohydrate (500 mg by mouth four times a day) is given while the catheter remains in place. The catheter is deflated at 5 to 7 days, removed if the site is stable, or refilled for another 3 to 5 days. Treatment with peroxide and bacitracin is continued until wound closure with a good cosmetic result.

A variety of options exist for fixation of unstable zygomatic arch fractures. A headcap or outrigger can be used to provide external traction with a transcutaneous bone screw; however, this is an inconvenient method. A coronal or preauricular (parotidectomy) approach with direct visualization and plating involves additional incisions and morbidity. Several authors use external splints made of oral airways, endotracheal tubes, aluminum finger splints, or molded acrylic laid over the fracture site. Several wires or sutures that are passed around the arch and splint support the zygoma.[4,5] Drawbacks include potential facial nerve injury with transcutaneous ties and pressure necrosis of skin beneath the splint. Foley catheters and various types of packing have been placed beneath the arch for support.[6]

The double balloon catheter offers a more attractive approach for several reasons. Placement is fast, is relatively simple, and uses the existing Gillies incision. The risks to the facial nerve and skin from pressure and transcutaneous sutures are avoided. The double balloon catheter has a shorter length and larger inflated surface than the Foley and is less likely to migrate from underneath the arch. Packing left in place 5 to 7 days has some risk of infection and may be painful to remove.

We highly favor this approach as a convenient, reliable method for supporting the unstable isolated zygomatic arch fracture.

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PEDIATRIC OTORHINOLARYNGOLOGY ENDOSCOPY DAYS

The First International Days in Pediatric Otorhinolaryngology Endoscopy will be held in Lyon, France, October 14-15, 1996. For further information, contact Marie-Claude Duchez, Departement d'ORL et de Chirurgie Cervico-Faciale, Hôpital E. Herriot, Place d'Arsonval, 69003 Lyon, France; fax 33 72 11 05 34.

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IMAGING CASE STUDY OF THE MONTH

PROSTATE CANCER METASTATIC TO THE ORBIT

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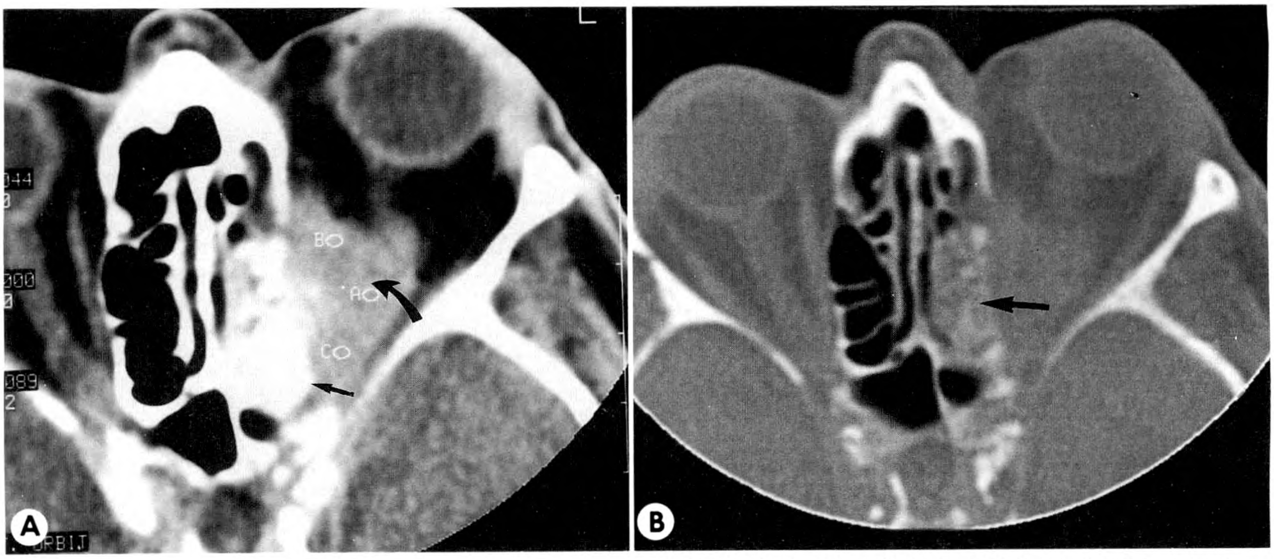
INTRODUCTION

Primary carcinoma of the paranasal sinuses is rare, accounting for approximately 0.3% of all cancer cases.[1] Metastases to the paranasal sinus are even less common, with fewer than 100 cases reported in the literature.[1] The most common tumor to metastasize to the paranasal sinuses is hypernephroma, followed by breast cancer, lung cancer, and seminoma.[2] Breast and lung cancer are the most common sources of orbital metastases (excluding leukemia and lymphoma).[3]

We present a patient with an orbital and ethmoid metastasis from the prostate gland with a characteristic osteoblastic appearance on computed tomography (CT) scan. One percent of patients with prostate cancer will have dissemination to the head and neck.[2] Reports of prostate cancer metastases to the paranasal sinuses are exceedingly rare.[4,5] Although prostate cancer metastases to the orbit are uncommon, the prostate is the third most commonly identified primary site for orbital metastases.[3] The evaluation of a man over 50 years old with an osteoblastic orbital lesion should begin with a thorough urologic evaluation rather than proceeding directly to an orbital biopsy.[3]

CASE REPORT

A 69-year-old man had a 2-month history of a gradual onset of diplopia, blurry vision, and pain in his left eye. He also had an 18-kg weight loss over the last year. Physical examination revealed proptosis and epiphora of the left eye. A CT scan showed an osteoblastic lesion in the septae of the left ethmoid sinus, with expansion into the left orbital apex (see Figure). Urologic workup was remarkable for a 30- to 35-g prostate with a firm ridge in the sulcus that appeared to extend to the side wall. A prostate-specific antigen test was performed and the antigen was markedly elevated at 69.80 ng/mL. A transrectal



Computed tomograms. A) Osteoblastic lesion involving superomedial wall of left orbit and left ethmoid sinuses (straight arrow). Associated soft tissue in left orbital apex (curved arrow). B) Bone mode axial computed tomogram showing blastic change of sinus septae with orbital wall destruction adjacent to orbital apex soft tissue mass (arrow).

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ultrasound test with prostate biopsy was positive for prostatic adenocarcinoma. To further delineate the extent of his disease, an endoscopic biopsy of the ethmoidal extension of the mass was done. This was positive for adenocarcinoma consistent with prostate carcinoma.

DISCUSSION

Prostate cancer is the second most common type of non-skin cancer in men.[6] Prostate cancer metastases occur in 70% to 90% of patients with prostate cancer.[3] Osseous metastases are the most common form of spread, with the most frequent sites being the pelvis, spine, femurs, and ribs, in order of decreasing frequency.[7] Sixty-six percent to 90% of the metastatic lesions are osteoblastic.[3] Flocks and Boatman[2] have shown than 1% (15/1,500) of patients with prostate cancer will have head and neck manifestations of the primary cancer. The most common site of dissemination is the lymph nodes (11/15).[2] The prevertebral venous plexus has been postulated as a route of spread to the head and neck.[1,5]

In our case the metastasis appears to be centered in the orbit, although it may have arisen from the lateral wall of the ethmoid sinus. There have been fewer than 20 cases of metastases to the ethmoid sinus reported, with only 1 case of prostate cancer metastatic to the ethmoids.[5] To our knowledge, 1 other case of prostate cancer metastatic to the paranasal sinuses (maxillary antrum) has been described.[4]

The prostate is the third most common primary site of cancer that metastasizes to the orbit.[3] In general, orbital metastases typically present with the rapid onset of proptosis, diplopia, redness, eyelid swelling, decreased visual acuity, ptosis, and epiphora.[3] Pain is a more common symptom in metastatic lesions than in primary orbital tumors.[3] Orbital metastases from prostate cancer differ from other orbital metastases in that the patients with prostate cancer have a much higher mean age than patients with other orbital metastases (70.1 years versus 53.6 years).[3] These lesions also differ in their radiographic appearance. Most orbital tumors present as osteolytic lesions or soft tissue masses, whereas prostate cancer metastases frequently present with osteoblastic changes.[3] Also included in the differential diagnosis for an osteoblastic lesion of the orbit would be osteoma, meningioma en plaque, Paget's disease, osteogenic sarcoma, osteomyelitis, fibrous dysplasia, ossifying fibroma, osteoblastoma, and neurofibromatosis.[3] Our case demonstrates that in a man over 50 years old with a rapidly progressive orbital mass and with osteoblastic changes on CT scan, prostate cancer must be ruled out. Evaluation should include a prostate-specific antigen test and thorough urologic examination rather than proceeding directly to an orbital biopsy.[3]

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AESTHETIC SURGERY OF THE FACE

The 8th Annual Symposium on Aesthetic Surgery of the Face will be held March 21-23, 1996, in San Francisco, California. For more information, please contact University of California, Office of Continuing Medical Education, 1855 Folsom St, MCB Room 630, San Francisco, CA 94143-0742; telephone (415) 476-4251; fax (415) 476-0318.

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IMAGING CASE STUDY OF THE MONTH

PEDIATRIC CRANIOCERVICAL NECROTIZING FASCIITIS

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INTRODUCTION

Craniocervical necrotizing fasciitis (CCNF) is a progressive, severe bacterial infection that involves the superficial musculoaponeurotic system (SMAS) and superficial fascial planes of the neck. Classically, the infection progresses rapidly, and the deep cervical fascial layers may become involved. Pathogenic organisms include group A β-hemolytic *Streptococcus, Staphylococcus aureus*, and obligate anaerobic bacteria.[1] These virulent bacteria, alone or in synergistic combination, produce a necrotizing infection of the fascial planes and soft tissues of the head, neck, and scalp. Early treatment is critical to prevent infectious spread to the great vessels or mediastinum that would produce life-threatening systemic toxicity and sepsis. The basis of successful treatment consists of early recognition of CCNF combined with aggressive surgical debridement and drainage of the involved fascial planes and tissues along with intensive patient support, and broad-spectrum intravenous antibiotic coverage.

CASE REPORT

A 4-year-old boy presented to the emergency room with a high-grade fever (40.5°C) and stiff neck. Twelve hours earlier he had been playing with a broom handle when he fell on it and injured his posterior pharyngeal wall. The patient had complained of a sore throat after the fall, but no violent coughing or vomiting was reported. He had no dyspnea or stridor. Physical examination revealed a small amount of purulent material in the oropharynx with no obvious lesions or asymmetry. The neck was very tender to palpation, and crepitance was present from the skull base to the intermammary line bilaterally. Breath sounds were clear and equal bilaterally. Oxygen saturation was 98% on room air. Laboratory studies revealed a white blood cell count of 29.3 with a left shift; other values were normal.

Craniocervical necrotizing fasciitis was the preliminary diagnosis, and intravenous Unasyn (ampicillin sodium–sulbactam sodium), clindamycin, and gentamicin were initiated. The operating room was notified and an emergent computed tomography scan was obtained. The Figure, A, shows air dissecting in the retropharyngeal and parapharyngeal spaces. The Figure, B, illustrates the extent of the air dissection around the visceral space and great vessels. The Figure, C, shows the infection extending into the mediastinum.

The patient was taken to the operating room emergently. Direct laryngoscopy revealed a 7-mm laceration of the posterior pharyngeal wall, with a small amount of purulent discharge. An apron incision was made and subplatsymal flaps were elevated. Air was present in the retropharyngeal space and in the carotid sheaths bilaterally. A thin exudate of "dishwater pus" was present in the tissues during the dissection. The carotid sheaths were opened bilaterally and the retropharyngeal space was bluntly dissected. The mediastinum was also dissected in the pretracheal plane. Cultures of the exudate were obtained from the oropharynx, retropharyngeal space, carotid sheaths, and mediastinum. Drains were placed into the retropharyngeal space bilaterally and the mediastinum. The wound was loosely reapproximated.

The patient was intubated for 48 hours and intravenous antibiotics were continued. He was afebrile and the white blood cell count decreased to 9.3 on postoperative day 2. All cultures grew *S aureus*. He continued 2 weeks on a regimen of Augmentin (amoxicillin–clavulanate potassium), recovering completely, without sequelae.

DISCUSSION

Craniocervical necrotizing fasciitis is a rare entity in the head and neck. In adults, CCNF is often associated with dental infections or trauma.[2,3] Recent interest has been stimulated by several articles in the lay press about "flesh-eating" bacteria that have

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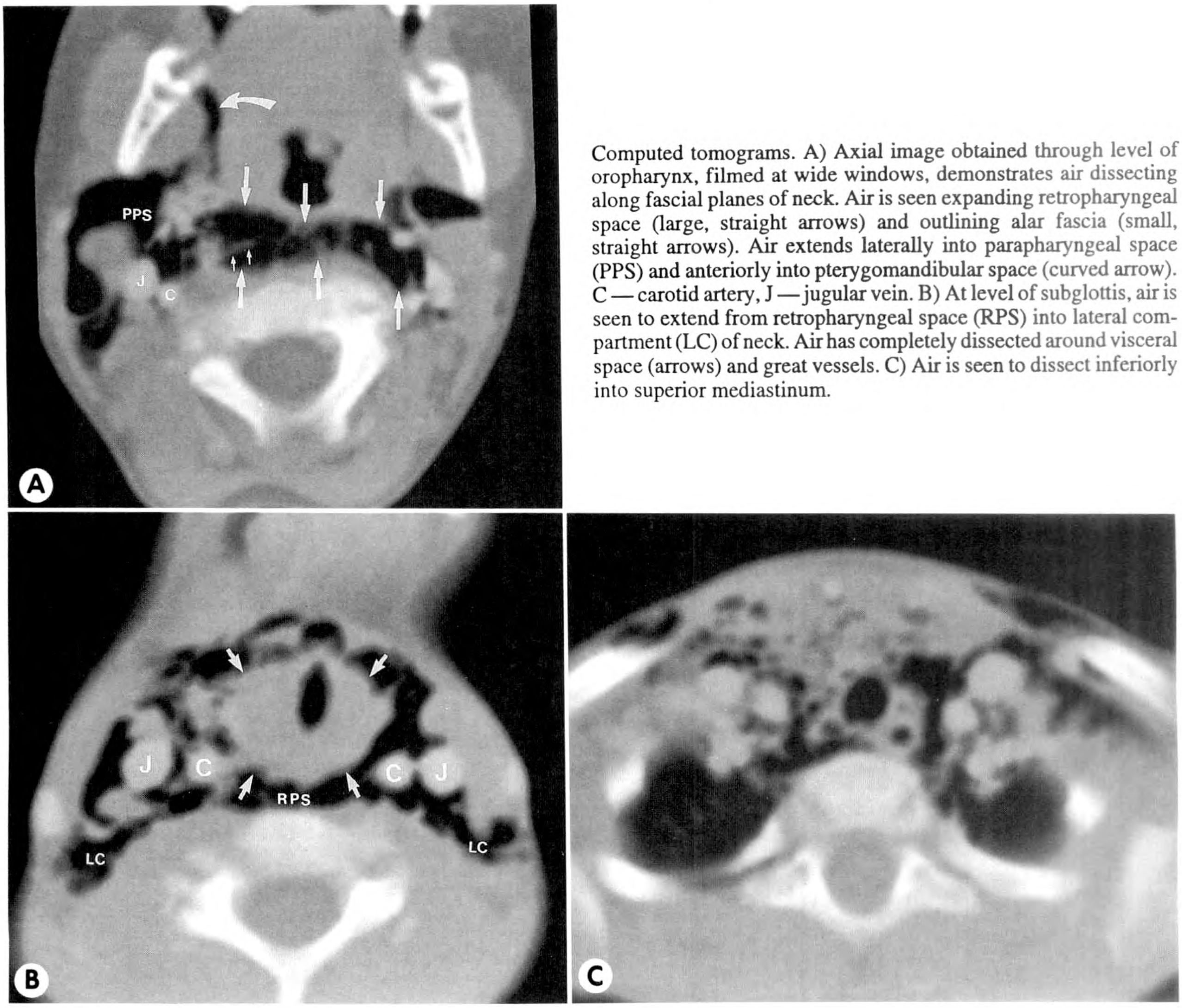

Computed tomograms. A) Axial image obtained through level of oropharynx, filmed at wide windows, demonstrates air dissecting along fascial planes of neck. Air is seen expanding retropharyngeal space (large, straight arrows) and outlining alar fascia (small, straight arrows). Air extends laterally into parapharyngeal space (PPS) and anteriorly into pterygomandibular space (curved arrow). C — carotid artery, J — jugular vein. B) At level of subglottis, air is seen to extend from retropharyngeal space (RPS) into lateral compartment (LC) of neck. Air has completely dissected around visceral space (arrows) and great vessels. C) Air is seen to dissect inferiorly into superior mediastinum.

caused several deaths in the United States and England. To our knowledge, this is the first reported case of CCNF in a pediatric patient. Other cases of necrotizing fasciitis in the pediatric population have typically involved the groin and trunk, occurring after surgical or birth trauma.[4]

The fascial plane initially involved in CCNF is the SMAS.[2,3,5] The SMAS invests the muscles of facial expression in a longitudinal sheath that is continuous with the platysma in the neck and the posterior aspect of the frontalis muscle in the face.[6] It is not completely understood why the SMAS fascia is the primary site of involvement. One theory is that the fascial planes' avascularity may favor the subcutaneous spread of infection, especially by anaerobic bacteria.[7] Complications of CCNF such as asphyxia, lung abscess, mediastinitis, or involvement of the great vessels are directly related to the spread of infection along the cervical fascial planes.

Early diagnosis is critical to the proper management of CCNF. Necrotizing fasciitis is a clinical diagnosis, and the physician must include CCNF in the differential diagnosis of all patients with severe head and neck infections. Initially the infection spreads along fascial planes with relatively little skin or muscle damage. There is pain, swelling, warmth, and erythema, which have been misdiagnosed as cellulitis or erysipelas. Soft tissue crepitance is another common finding. Hyperpyrexia, tachypnea, tachycardia, lethargy, and apathy are associated features often seen in CCNF.

Computed tomography of the head, neck, and superior chest is the imaging modality of choice if the diagnosis is uncertain or if the feasibility of surgical intervention needs to be assessed. It gives information on the extent of the disease, confirms the presence or absence of gas, helps localize the initial site of infection, and provides anatomic information to help guide the surgical debridement.

Craniocervical necrotizing fasciitis is an aggressive infection of the cervical fascia caused by particularly virulent bacteria alone or by the synergistic activity of aerobic and anaerobic organisms. Early diagnosis is essential to optimize survival and minimize morbidity. The diagnosis is based on clinical evidence, and definitive surgical treatment should not be delayed while laboratory or radiologic studies are completed. Therapy is based on aggressive surgical debridement, repeated as required, broad-spectrum intravenous antibiotic coverage, intensive care unit support, and treatment of associated metabolic and electrolyte abnormalities.

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EPITHELIAL HYPERPLASTIC LESIONS OF THE LARYNX

The International Conference on Epithelial Hyperplastic Lesions of the Larynx will be held October 28-30, 1996, in Ljubljana, Slovenia. For more information, contact Bostjan Luzar, MD, or Aleksandar Vodovnik, MD, Institute of Pathology, Korytkova 2, 61105 Ljubljana, Slovenia; telephone 386 61 1403042 (Int. 268); fax 386 61 301 816; E-mail Vodovnik@IBMI.MF.UNI-LJ.SI.

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CLINICOPATHOLOGICAL CONSULTATION

BASALOID SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK: CLINICOPATHOLOGICAL FEATURES AND DIFFERENTIAL DIAGNOSIS

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Basaloid squamous cell carcinoma is a recently recognized variant of squamous cell carcinoma. The lesion is histologically distinctive and manifests a predilection for the supraglottis, pyriform sinus, and tongue base. The immunohistochemical profile is discussed. The differential diagnoses include adenoid cystic carcinoma, small cell neuroendocrine carcinoma, basal cell adenocarcinoma, adenosquamous carcinoma, squamous cell carcinoma, spindle cell squamous carcinoma, mucoepidermoid carcinoma, and adenoid squamous cell carcinoma. Basaloid squamous cell carcinoma is a biologically high-grade tumor with a propensity for nodal as well as systemic metastases. It is a morphologic and phenotypic entity with a separate prognostic significance.

Basaloid squamous cell carcinoma (BSCC) is an aggressive, recently recognized, biphasic variant of squamous cell carcinoma (SCC) that was first identified and accurately described in the head and neck by Wain et al[1] in 1986. The World Health Organization's classification has included this tumor in its revised edition of *Histological Typing of Tumours of the Upper Respiratory Tract and Ear*.[2] It is not, however, unique to this area of the body. Similar, if not identical, tumors have also been described in the esophagus, lung, uterine cervix, and anus.[3-8]

Other terms have been used to designate this phenotype, such as basaloid carcinoma, adenoid basal cell carcinoma, carcinoma with adenoid cystic differentiation, adenoid cystic–like carcinoma, etc. When the tumor occurs in the anal canal, it has been called cloacogenic carcinoma.

Many papers have been published since the first report, and the incidence of this tumor is constantly increasing.[1,4,9-31] The tumor occurs in both sexes, but predominantly in men in their 60s and 70s. The lesion has a predilection for the supraglottis, pyriform sinus, and tongue base. Cases have also been reported in other endolaryngeal sites,[1,4,10,12,13,19,26] as well as in the floor of the mouth, tonsil, nasal cavity, paranasal sinuses, nasopharynx, palate, and buccal mucosa.[13,14,17,20-22,27-29] In rare instances, it may even present as an unidentified primary with cervical lymph node metastasis.[13,23,27]

The tumor may exhibit a multifocal mucosal origin, although this has not been common in our experience. Seidman et al[12] indicate that BSCC may also be associated with a high incidence of second primary neoplasms (synchronous or metachronous) of the upper aerodigestive tract. They report 2 cases of BSCC, arising in the pyriform sinus and vallecula, respectively, each of which was associated with a second primary — a small cell carcinoma of the esophagus in the former and an epidermoid carcinoma of the palate in the latter. McKay and Bilous[9] also describe a BSCC of the pyriform sinus associated with an SCC of the left arytenoid, and Wan et al[21] report a BSCC of the nasal cavity in a patient who also had a nasopharyngeal carcinoma. Of the 40 patients with BSCC reported by Banks et al,[13] 2 had additional malignancies: 1 had chronic lymphocytic leukemia and the other had adenocarcinoma of the colon and the prostate. Symptoms depend on the site of the lesion, and cervical metastases are often detectable on presentation.

It may be that BSCC is etiologically linked to tobacco and alcohol abuse.[27] The lesion seems to originate from a totipotent primitive cell located in the basal cell layer of the surface epithelium, or within seromucous glands.[1]

Macroscopically (Fig 1), the tumor usually appears as a white or yellow-white, firm, poorly defined, exophytic, polypoid, and centrally ulcerated

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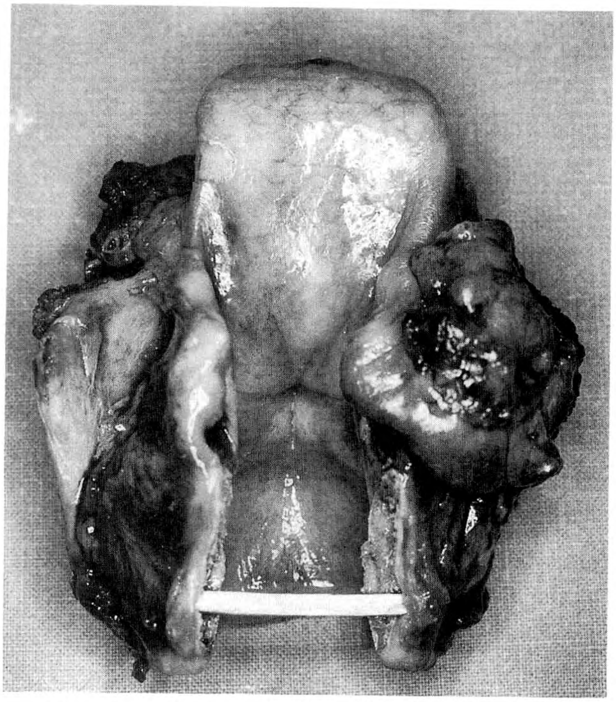

Fig 1. Gross photograph of basaloid squamous cell carcinoma. Ulcerating tumor involving right aryepiglottic fold and pyriform fossa.

mass with peripheral submucosal infiltration. As a result of the latter, it is often clinically mistaken as being of nonepidermoid origin. It may range from 1 to 6 cm in greatest dimension.

Microscopically, it has two components, ie, basaloid (Fig 2) and squamous cells (Fig 3). The basaloid cells have increased nuclear-to-cytoplasmic ratios and scant pink to amphophilic cytoplasm. The nuclei are typically round and vary from hyperchromatic to moderately vesicular, with or without nucleoli. The basaloid cells grow as smooth-contoured lobules, solid masses, or cords of cells, frequently with mitoses and a prominent peripheral palisading of nuclei. Many of the lobules exhibit central (comedo) necrosis and occasionally contain ducts or cystic-like spaces with stromal mucinous material. In other areas, the basaloid cells are separated by dense pink acellular hyaline material or surround hyaline stromal cores. In rare instances, BSCC may also exhibit a malignant spindle cell component much like spindle cell carcinoma.[29,30,32]

Although the basaloid component predominates, a definable element of SCC must be present for the diagnosis to be made with confidence: the squamous carcinoma may be in situ or invasive and is typically well to moderately differentiated. If there is extensive surface ulceration, only dysplastic changes may be seen in the intact epithelium. It is not infrequent for the basaloid islands to exhibit squamous differentiation, and when this occurs, the transition between the two components may be abrupt (Fig 4), or there may be a narrow zone of transition. Metastases may contain basaloid cells, squamous cells, or both (Fig 5). Most, however, are basaloid in composition. This tumor has also occasionally been diagnosed by fine-needle aspiration cytology.[31]

Cytokeratin immunoreactivity in BSCC is a constant finding in reported cases,[4,11,13,16,22,23] although the percentage of positive cells in the basaloid component varies greatly among different reports. The same antibodies gave different results in different laboratories. This may be due to a different sensitivity of the immunohistochemical method involved or to different specimen fixation times (antigenicity is impaired by prolonged fixation). Antigen retrieval methods could render cytokeratin immunohistochemistry more reproducible between different laboratories. It is generally agreed that not all the basaloid cells stain with cytokeratin antibodies and that basaloid cells never show punctate or dot staining with cytokeratin. It is therefore prudent to use a cocktail of keratin antibodies (clones CAM 5.2, AE1-AE3, and 34βE12 are the most frequently employed in this setting) to avoid false-negative results. The epithelial nature of this neoplasm is further substantiated by epithelial membrane antigen and carcinoembryonic antigen immunoreactivity. Vimentin immunoreactivity was reported, with positive results in 1 of 3 cases, by Tsang et al[4]; all cases were negative in the series reported by Klijanienko et al.[23] These results contrast with our experience, in which most BSCC cases displayed vimentin immunostaining in the vast majority of the neoplastic cells (basaloid component). Such different results are probably explained by differences in sensitivity of the immunoperoxidase method employed, because all investigators (including our group) used the same antibody (clone V9, from Dako). The pattern of vimentin immunoreactivity in basaloid cells is peculiar, forming a delicate perinuclear rim, frequently with a small dot. Glial fibrillary acidic protein has been tested in BSCC with negative results; immunoreactivity with other intermediate filaments, such as neurofilaments and desmin, has not been reported. Actin was reported as focally positive in 4 of 9 cases by Tsang et al,[4] but completely negative in 35 cases described by Banks et al,[13] using the same antibody (HHF35). We observed focal actin staining in a small proportion of our cases, using two different anti-actin antibodies, HHF35 and 1A4. Studies for S-100 protein have produced divergent results in the hands of different groups.[4,13,22,23] In our series, focal S-100 immunoreactivity was observed in basaloid cells in few cases; most displayed numerous S-100–positive dendritic cells intermingled with

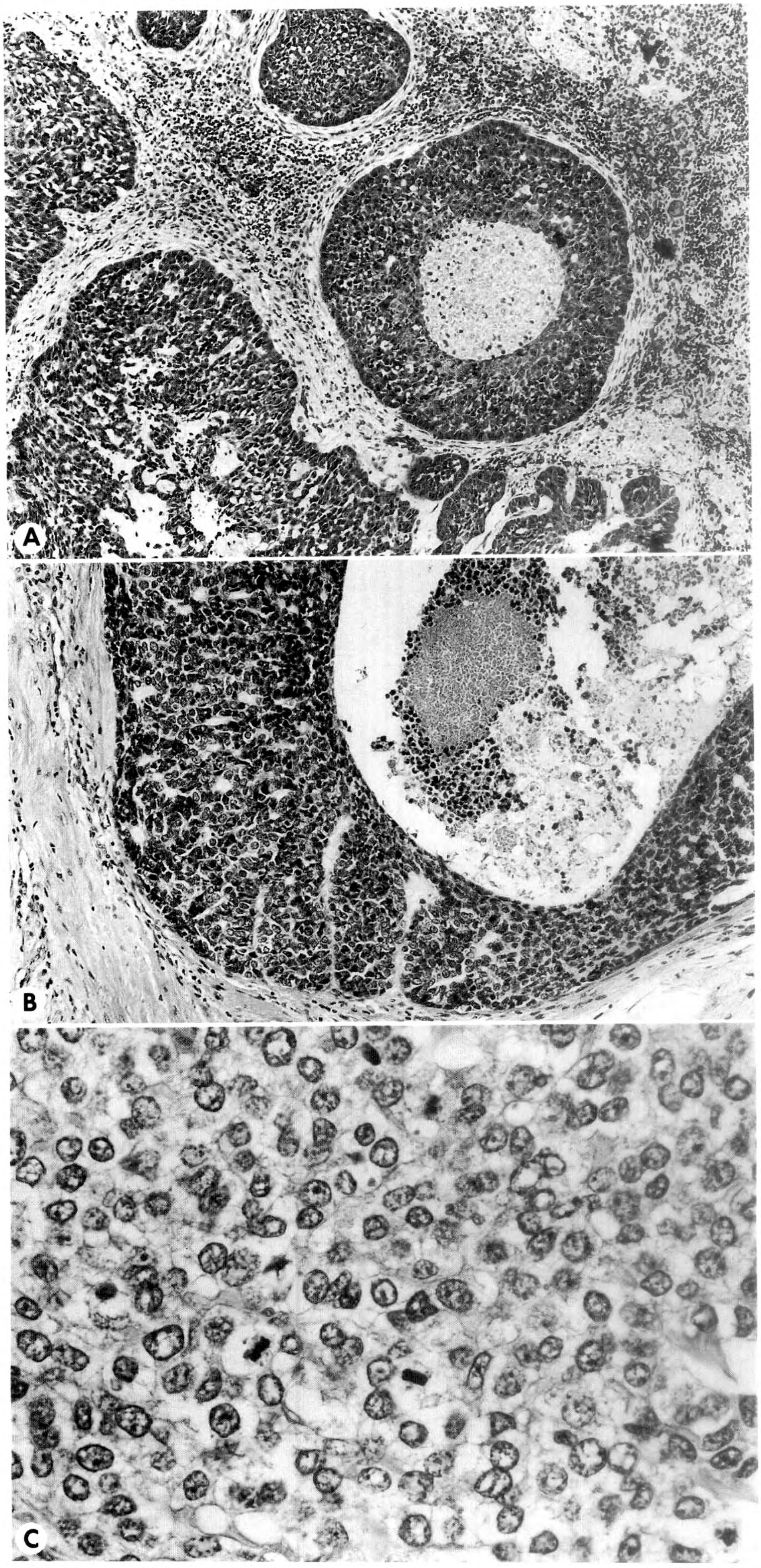

Fig 2. Basaloid component. A) Low magnification showing smooth-contoured lobules of basaloid cells. Note tendency of nuclei to palisade at periphery. B) High magnification showing details of basaloid cells. Note central (comedo) necrosis. C) High magnification showing details of basaloid neoplastic cells and prominent mitoses.

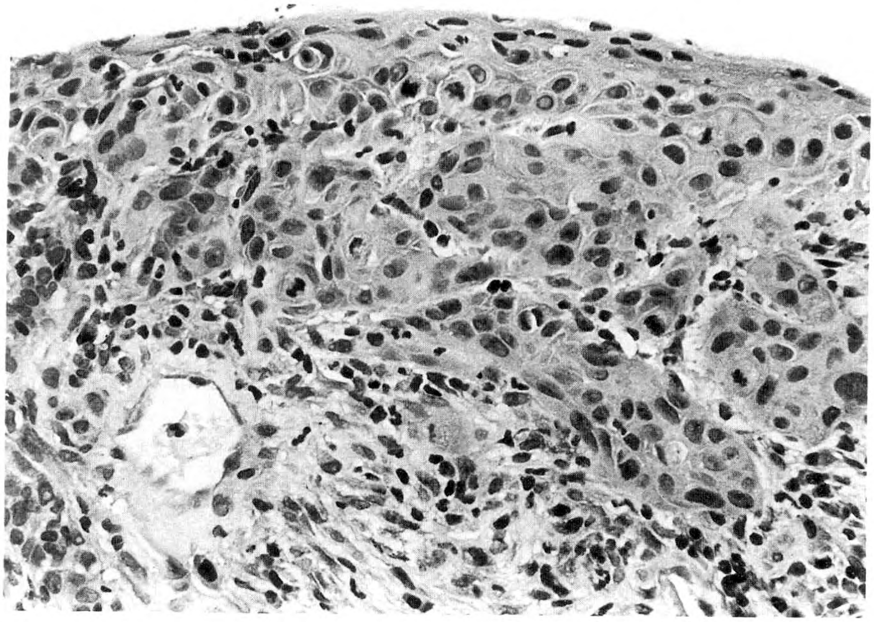

Fig 3. Mucosal component showing focus of superficially invasive squamous cell carcinoma.

the neoplastic population. Apart from the positivity for neuron-specific enolase reported in the literature,[11-13,23] all reliable neuroendocrine markers, such as chromogranin and synaptophysin, have been reported as negative.

Whether determining the DNA content of the tumor has prognostic significance remains controversial. Luna et al[10] studied 9 BSCCs and found that 5 were aneuploid and 4 were diploid. Three of the 4 patients with diploid carcinomas died with distant metastases 11 to 20 months after diagnosis, whereas 2 of the 5 with aneuploid tumors died of their disease at 36 and 43 months. The authors concluded that patients with aneuploid BSCC had a better mean survival time (39.5 months) than those with diploid tumors (16.3 months). Raslan et al[27] examined 10 BSCCs by flow cytometry and found 6 diploid and 4 aneuploid cases. Five of the 6 patients with diploid tumors and all 4 patients with aneuploid tumors developed early regional and distant metastases. Two of the 10 patients died: 1 with an aneuploid tumor at 4 months and 1 with a diploid tumor at 3.5 years. The authors concluded that tumor ploidy provided no further information by comparison with the findings of routine hematoxylin and eosin evaluation.

Ultrastructurally, basaloid cells contain desmo-

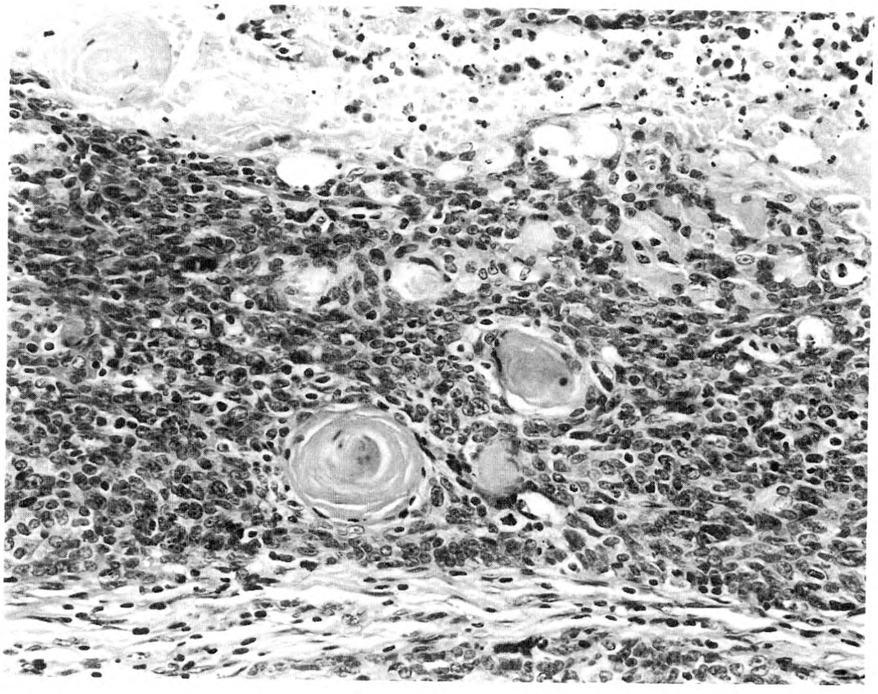

Fig 4. Basaloid squamous cell carcinoma showing abrupt keratinization (keratin pearls). Tumor necrosis is apparent at top.

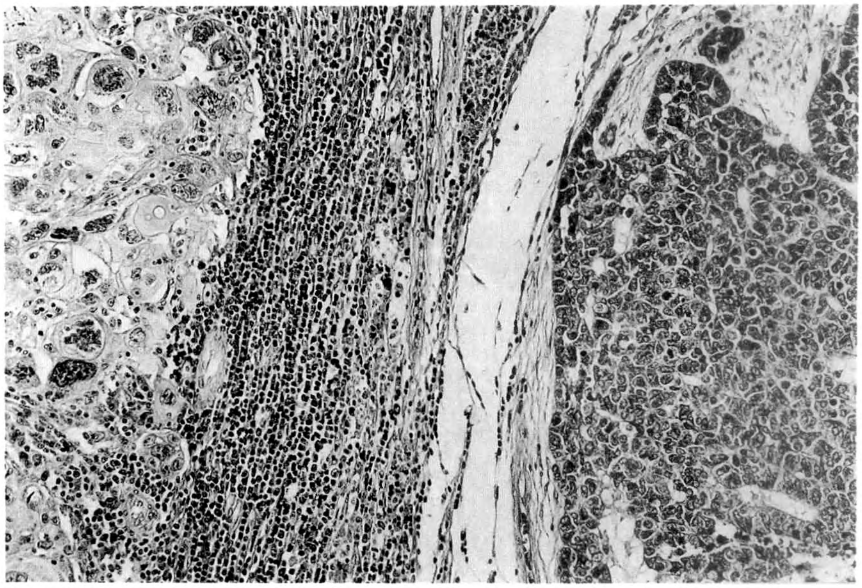

Fig 5. Cervical lymph node showing squamous (on left) and basaloid (on right) neoplastic components.

somes, rare tonofilaments, and free ribosomes, but few other organelles. Neurosecretory granules, myofilaments, and secretory granules are absent.[1] The squamous cells are characterized by typical desmosomes and tonofilaments.

Because of its heterogeneous composition, it is often difficult, or even impossible, to confidently establish a diagnosis of BSCC on small biopsy specimens. The criteria are as follows[32]:

1. Patients are usually in their 60s or 70s.
2. The supraglottis, pyriform sinus, and tongue base are the sites of predilection.
3. There is an ulcerated, exophytic tumor with submucosal soft tissue infiltration.
4. There is the simultaneous presence of a basaloid component and a squamous cell carcinoma, which may be in situ or invasive (well- or moderately differentiated).
5. The basaloid component is composed of solid

COMPARISON OF PATHOLOGIC AND CLINICAL FINDINGS BETWEEN BASALOID SQUAMOUS CELL CARCINOMA AND ADENOID CYSTIC CARCINOMA

Features	*Basaloid Squamous Cell Carcinoma*	*Adenoid Cystic Carcinoma*
Sex	Male predominance	No significant sex predominance
Age	50-70 years	40-60 years
Sites of predilection	Supraglottis, pyriform sinus, tongue base	Major salivary glands, palate, sinonasal tract
Classic cribriform pattern	Absent	Present
Mitoses	Abundant	Scarce
Nuclear pleomorphism	Prominent	Rare
Squamous component	Present	Absent
Perineural growth	Inconstant	Prominent
Osseous or chondroid metaplasia	May be present	Absent
Cervical lymph node metastases	Usually present	Absent
Necrosis	Frequent	Rare
Clear cells	May be present	Absent
Vimentin positivity	Perinuclear rim and dot	Diffuse cytoplasmic
Actin positivity	Focal	Diffuse
S-100 positivity	Focal; dendritic cells present	Diffuse; dendritic cell absent
Collagen IV	Absent	Abundant
Ki67/MIB1	High	Low
Presentation	High-stage	Low-stage
Clinical course	Very aggressive	Slow, indolent
Short-term therapeutic results	Unfavorable	Favorable

Modified from Ferlito.[32]

growths of cells in a lobular configuration, closely adjacent to the surface mucosa; the cells are small, crowded, with scant cytoplasm and dark nuclei, with or without nucleoli; small cystic spaces contain material that may be positive with periodic acid–Schiff stain and Alcian blue.

6. Coagulative necrosis is often present in the central areas of the tumor lobules (comedonecrosis).
7. There are foci of hyalinization of the stroma.
8. Mitotic figures are numerous.
9. Lymphovascular permeation is common.
10. Peripheral palisading is prominent.
11. Clear cells, large cells with vesicular nuclei, angiogenesis, desmoplasia, neurotropism, a spindle cell component with tumor giant cells, a small cell component, or osseous or chondroid metaplasia may also be present.
12. The tumor shows highly metastatic behavior.

The differential diagnosis includes adenoid cystic carcinoma (ACC), small cell neuroendocrine carcinoma, basal cell adenocarcinoma, adenosquamous carcinoma, SCC, spindle cell squamous carcinoma, mucoepidermoid carcinoma, and adenoid squamous cell carcinoma.

Adenoid cystic carcinoma histologically exhibits three patterns of growth: tubular, cribriform, and solid. It is the solid variant of ACC, composed of small basaloid cells — coupled with the fact that 60% to 70% of cases arise in minor salivary glands — that poses the most difficulty.[33,34] Knowledge of the clinical findings can be useful. For instance, BSCC is more often associated with positive cervical lymph nodes, whereas ACC is not. In ACC there are no signs of invasive SCC, carcinoma in situ, dysplasia, or focal squamous differentiation, all of which occur in BSCC.[32] Mitoses, nuclear pleomorphism, and necrosis are more prominent in BSCC and rare in ACC.[27] The Table[32] summarizes the different pathologic and clinical aspects of the two tumors.

Klijanienko et al[23] have suggested that ACC and BSCC can be distinguished by using immunohistochemical stains for vimentin and S-100 protein. In their evaluation of 12 BSCCs, they found that the BSCCs were uniformly negative for these markers, while ACCs were positive. The utility of S-100 protein staining in this distinction is limited, however, since 39% of the BSCCs reported by Banks et al[13] were S-100 protein–positive. Focal positivity in the neoplastic cells for S-100 protein, and also for muscle-specific actin, has been observed in our unpublished series and has also been reported in the literature.[4,13]

Vimentin immunoreactivity may be present in ACC and in BSCC, but in the former the positivity is diffuse in the cytoplasm, whereas in the latter it is present as a delicate perinuclear rim with a small dot. Hyaline material is decorated by type IV collagen in ACC, whereas similar material is negative in BSCC.

Small cell neuroendocrine carcinoma similar to the type seen in the lung can occur in the head and neck, and when it does, the larynx is one of the most common sites.[35,36] Negativity for the neuroendocrine markers (eg, chromogranin, Leu-7, synaptophysin, protein gene product 9.5) and the absence of neurosecretory granules observable by electron microscopy exclude the possibility of a neuroendocrine carcinoma.[32] In addition, vimentin immunoreactivity is not usually observed in neuroendocrine carcinoma. This differential diagnosis carries crucial therapeutic implications.

Basal cell adenocarcinoma is another recently recognized tumor of the head and neck that occurs mainly in the parotid gland (80% to 90% of cases) and rarely in the submandibular gland or minor salivary glands. It is generally regarded as the malignant counterpart of basal cell adenoma, although in practice it most often arises de novo, only occasionally (10%) deriving from a preceding basal cell adenoma.[37,38] In about 10% of cases, it is associated with cutaneous tumors, especially dermal eccrine cylindromas.[38] Unlike BSCC, which is an aggressive life-threatening neoplasm with frequent regional and distant metastasis, basal cell adenocarcinoma rarely results in metastases or death. Basal cell adenocarcinoma can be distinguished from BSCC by its site of origin (parotid gland) and the absence of metastases.

Adenosquamous carcinoma demonstrates separate foci of adenocarcinoma in addition to a squamous cell component,[39,40] whereas the former is not seen in BSCC. In addition, stains for epithelial mucin are strongly positive in adenosquamous carcinoma, and either negative or only weakly positive in BSCC.

Stroma hyalinization is not a feature of SCC, but is prominent in BSCC. Also, the pattern of vimentin immunoreactivity is different in SCC and BSCC: in the infrequent cases of SCC the vimentin diffusely stains the entire cytoplasm, while in BSCC the positivity is characterized by a delicate perinuclear rim with a small dot. A basaloid pattern is not a feature of spindle cell squamous carcinoma, mucoepidermoid carcinoma, or adenoid squamous cell carcinoma.

With a 38% mortality rate at 17 months' median follow-up,[27] BSCC is highly malignant. Sixty-four percent of patients either already have or will develop positive cervical lymph nodes, and 44% will have distant metastases at some stage of their disease, particularly to the lungs, liver, bones, brain, and skin.[27]

Surgery, including cervical lymph node dissection, and postoperative irradiation appear to be the treatment of choice. The high incidence of distant metastases suggests that adjuvant chemotherapy may also be warranted.[9,10] Although BSCC appears to be more lethal than conventional SCC, Luna et al[10] indicate that when the two tumors are matched for anatomic site, stage, and treatment they actually have comparable survival rates. These authors' data were based on a very small series of BSCCs, however, and need further verification. A report from Coppola et al[22] indicates that BSCC of the floor of the mouth is an aggressive neoplasm and is prognostically worse than conventional SCC, regardless of the grade of the latter.

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FUNCTIONAL ENDOSCOPIC SINUS SURGERY: BASIC AND ADVANCED TECHNIQUES

The course Functional Endoscopic Sinus Surgery: Basic and Advanced Techniques will be held in Graz, Austria. The English course will be held July 11-13, 1996, and the German course September 5-7, 1996. For further information, contact Prof H. Stammberger, MD, ENT Department, University Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; fax ++43 316 385 3425.

LETTERS TO THE EDITOR

To the Editor:

I would like to comment on the recent paper by Pollak et al (Are Papillary Adenomas Endolymphatic Sac Tumors? *Ann Otol Rhinol Laryngol* 1995;104:613-9). The authors report on a case of bilateral aggressive papillary tumors of the temporal bone in a von Hippel–Lindau disease patient. By virtue of surgical observations and immunohistochemical evaluation, the authors conclude that the tumor in their case report was not derived from endolymphatic sac or duct epithelium, but rather originated from the mucosal lining of the temporal bone. Although the authors have extensively documented the clinical course and radiographic progression of the tumor as well as its pathology, I disagree with their conclusion with regard to the origin of this lesion.

Features of this patient's history that clearly support endolymphatic sac or duct origin are numerous. Most important, the patient has a clinical history of progressive, fluctuating sensorineural hearing loss, vertigo, and tinnitus as well as lateral canal paresis. These symptoms are clearly congruent with endolymphatic hydrops, which is expected when the endolymphatic duct is blocked and is also expected during the evolution of a primary endolymphatic duct or sac tumor. The ability of aggressive papillary tumors of the temporal bone to originate within the endolymphatic sac and duct has clearly been demonstrated in a recent publication.[1] In the accompanying temporal bone histomicrograph from a similar case described in that article, the authors demonstrate a primary endolymphatic space tumor filling the confines of the endolymphatic duct and only partially filling the lumen of the sac. Clearly, if this patient were operated on and the distal aspect of the sac were opened, it would also fail to demonstrate tumor, as was observed by Pollak et al. However, if the sac was followed to the duct and behind the posterior semicircular canal, as would be necessary for accurate documentation of an endolymphatic duct tumor's origin, one would find a tumor filling and obliterating the duct.

The computed tomography scan that Pollak et al provided clearly shows destruction of the posterior semicircular canal, as would be expected from an endolymphatic sac or duct tumor that originated from duct epithelium. However, the authors hypothesized that the tumor began in the epithelium of the mucosal lining of the temporal bone. Pathophysiologically, such a tumor would not create endolymphatic hydrops, which is certainly the most common cause of the symptoms that this patient so clearly demonstrated early in the course of her disease.

Pollak et al also evaluated the histochemical profile of their patient's tumor using antibodies to cytokeratin, vimentin, S-100 protein, glial fibrillary acidic protein (GFAP), and chromogranin. Because of the lack of positive stains for vimentin, S-100, GFAP, and chromogranin, they conclude that the tumor originated from middle ear mucosa, which has a similar immunohistochemical fingerprint. However, comparison of the author's case with a similar tumor removed by Hassard et al[2] and reviewed by Heffner[3] reveals significant similarities to the tumor evaluated by Pollak et al. Specifically, both tumors stained strongly positive for cytokeratin. Both cases were negative for S-100. Both were positive for LU-5. The only differences lie in a lack of staining for GFAP in the authors' case. Vimentin was not tested for in Hassard's case. Therefore, the main immunohistochemical difference between those two cases is that of a lack of staining for GFAP. Because of the fact that a controlled immunohistochemical analysis of these rare tumors has not yet been performed, it is impossible to rule in or rule out endolymphatic sac tumor by virtue of immunohistochemical variations at this time. To date, immunohistochemical data regarding endolymphatic sac tumors come from sporadic case reports from different centers utilizing antibodies from various sources. Therefore, immunohistochemical data are insufficient at this time for use as a screening tool of potential endolymphatic sac tumors. Controlled studies and detailed analysis of pooled tissue samples will be necessary before relying on immunohistochemical data as a determinant of tumor origin.

Finally, the third criterion that the authors use in an attempt to distinguish the aggressive papillary tumor removed from their von Hippel–Lindau patient was the presence of kinocilia. They note that kinocilia are absent from endolymphatic sac epithelium in a number of studies and conclude that the tumor from their patient must come from middle ear mucosa, which is ciliated.

However, ultrastructural studies by Schuknecht[4] and Qvortrup et al[5] clearly demonstrate kinocilia in endolymphatic sac and duct epithelium, respectively. It is likely that the tumor identified by Pollak et al began in the endolymphatic duct, producing symptoms of hydrops, and then progressed anteriorly through the posterior semicircular canal and inferiorly to the top of the jugular bulb. A reexamination of this case using this information supports its origin within the endolymphatic system and warrants continued surveillance of such tumors in patients with von Hippel–Lindau disease.

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To the Editor:

Thank you for the opportunity to reply to Dr Megerian's comment on our paper on papillary adenomas of the temporal bone. Dr Megerian states that the clinical symptoms of our patient clearly support endolymphatic sac or duct origin. Although the time course of the sensorineural hearing loss was Meniere's-like, we do not agree that the observed symptoms "are clearly congruent with endolymphatic hydrops, which is expected when the endolymphatic duct is blocked and is also expected during the evolution of a primary endolymphatic duct or sac tumor." The occlusion of the endolymphatic sac and duct induces endolymphatic hydrops consistently in guinea pigs, frequently in rabbits and cats, rarely in chinchillas, and not at all in squirrel monkeys.[1] It remains questionable whether humans behave in this respect more like guinea pigs than monkeys. Furthermore, Megerian himself states in his paper that in the ear with a papillary proliferative epithelial lesion within the confines of the endolymphatic sac "the cochlea...had no evidence of endolymphatic hydrops." [2(p803)] Clinically, Megerian's patient suffered from a mild high-frequency sensorineural hearing loss in that ear. The hearing loss in other patients with von Hippel–Lindau disease and papillary adenoma of the temporal bone was described 1) as steadily progressive over 8 and 9 years[3,4]; 2) as severe bilateral loss since infancy[5]; and 3) as fluctuating, deteriorating to profound sensorineural hearing loss after 1 year (right ear), and as sudden, followed by fluctuating thresholds and a complete loss of hearing after a transmastoidal tumor excision in an attempt to preserve hearing 5 months later (left ear).[6] The onset of the auditory symptoms in our patient occurred long before radiologic signs of involvement of the endolymphatic sac or duct region by the tumor. Therefore, interference with cochlear blood flow secondary to tumor blockage of the inferior cochlear vein above the jugular bulb may also be at the origin of the observed symptoms.

In the course of surgery (subtotal petrosectomy with removal of the otic capsula[7]) the endolymphatic sac was followed to the duct and behind the posterior semicircular canal to the vestibule (as necessary for accurate documentation of an endolymphatic duct tumor's origin), without finding a tumor filling and/or obliterating the duct. After opening the vestibule, blood was found draining from the endolymphatic duct. The inner ear spaces were filled with xanthochromic fluid. The tumor clearly arose from the top of the jugular bulb.

We do not agree with Megerian's statement that the computed tomography scan provided in our paper clearly shows destruction of the posterior semicircular canal. For limitations of space, no computed tomography scans at the level of the posterior semicircular canal have been shown in our paper (see also diagram in Fig 2). Radiologic signs of invasion in this canal were first noticed in the third year.

In contrast to that of Dr Megerian, our opinion is that the immunohistochemical profile of our case is not similar to those found by Heffner[8] in Hassard's case, as we have discussed in our paper. The positive reaction in Hassard's case for cytokeratin and glial fibrillary acidic protein and the negative one for S-100 protein led us to the conclusion that this pattern is not characteristic of neuroectodermal or ectodermal histogenesis (page 618 of our article, *Ann Otol Rhinol Laryngol* 1995;104:613-9).

Megerian's arguments against our statement of absent kinocilia in the endolymphatic sac are based on the finding of one single kinocilium in the endolymphatic duct in the rat[9] and a rudimentary single kinocilium in a human endolymphatic sac reported in the 2nd edition of Schuknecht's *Pathology of the Ear*. The "lawn" of kinocilia found in our tumor (Fig 4) differs significantly from the mentioned rudiments, but compares well with the ciliated epithelial lining of the human mastoid.

Finally, we agree with Megerian's last statement that patients with von Hippel–Lindau disease warrant continued surveillance of these tumors — not primarily because the origin of these tumors is in the endolymphatic sac (papillary adenomas of the temporal bone may be of different origin), but because 1) patients with von Hippel–Lindau disease affected by a papillary adenoma of the temporal bone have a more than 50% risk of having a contralateral tumor and because 2) these tumors are locally aggressive and therefore should be treated by radical surgery.

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BOOK REVIEW

Infections in Childhood. Ear, Nose and Throat Aspects

Jacob Sadé, editor. Hard cover, illustrated, indexed, 460 pages, 1994. Excerpta Medica, Amsterdam, the Netherlands, $200.

This book consists of papers from the International Conference in Pediatric Otorhinolaryngology held in Jerusalem in November of 1993. In substance and format, the volume represents a continuation (review) of clinically significant discussions relevant to the many facets of pediatric otorhinolaryngology.

Inflammation is the main subject. The subject matter is briefly represented. It is refreshing to read research expositions that are easy to comprehend. Notwithstanding the mass of publications dealing with this meeting (337 papers, 12 panels, 617 participating otolaryngologists), there is much rehash of old material in this volume that, on one hand, gives excellent reviews, and on the other hand, sometimes consists of a "report of what I am doing." There is enough innovative material, such as an idea for a new ossicular prosthesis; new information concerning gaseous partial pressures and total pressures in the middle ear, including mechanisms of negative pressure and an explanation of the pathogenesis of primary acquired cholesteatomas.

In substance and format, the volume represents, for the most part, a continuation of clinically significant discussions relevant to the many facets of pediatric inflammatory otorhinolaryngology. Each of the 88 chapters is written in a concise, clear, well-organized manner.

Dennis G. Pappas, MD
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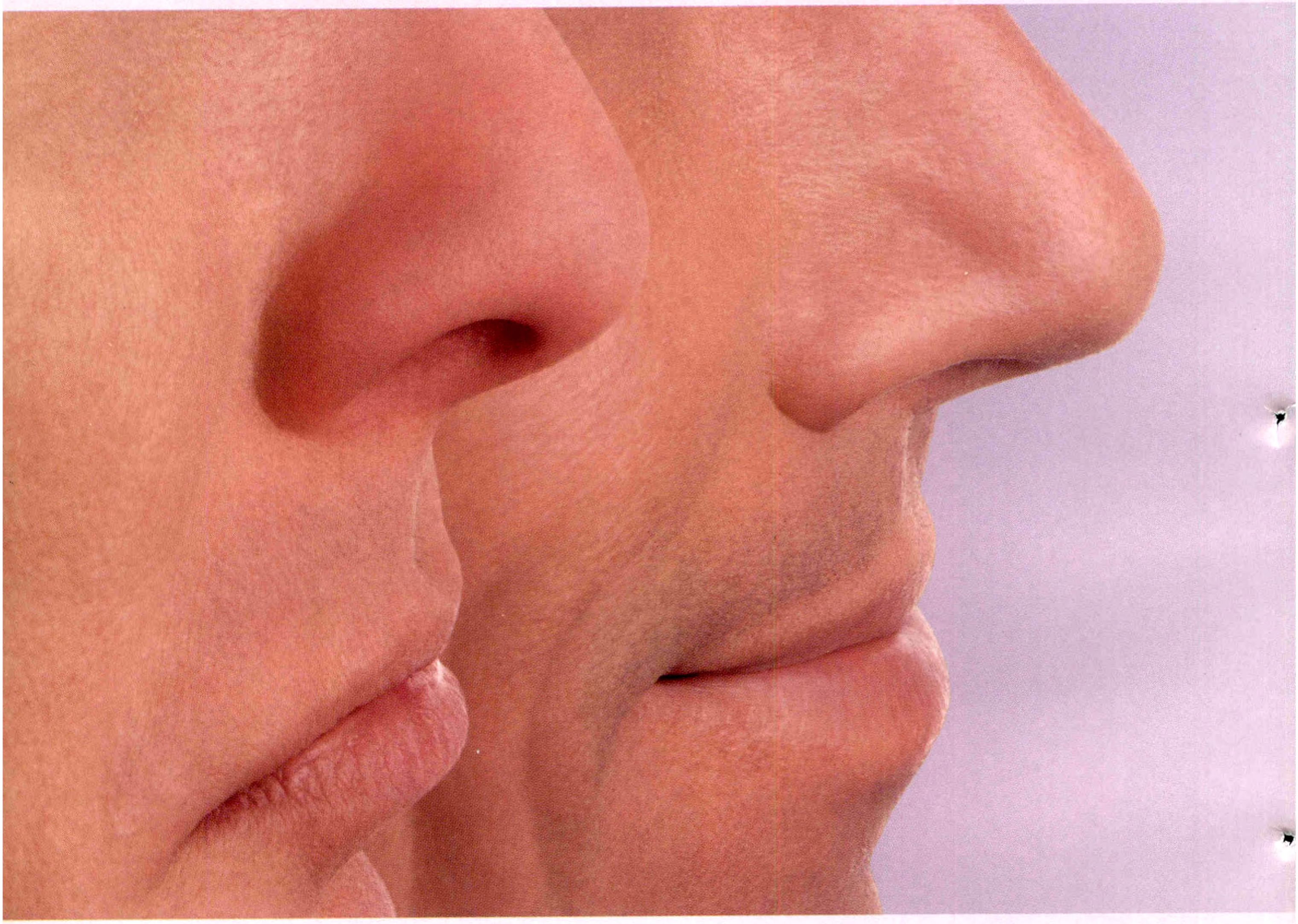

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VOCAL FOLD PARALYSIS FOLLOWING THE ANTERIOR APPROACH TO THE CERVICAL SPINE

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The anterior cervical approach is commonly used for access to the cervical spine. Vocal fold paralysis (VFP), a complication of this approach, is underrepresented in the literature. A review of the database of the Vanderbilt Voice Center revealed 289 patients with VFP, including 16 patients who developed paralysis as a result of an anterior cervical approach. The paralysis was on the right side in all but 1 patient. Compared to patients who developed VFP after thyroidectomy and carotid endarterectomy, patients with VFP after an anterior cervical approach have a higher incidence of aspiration and dysphagia, suggesting the presence of trauma to the superior laryngeal and pharyngeal branches as well as the recurrent branch of the vagus nerve. Two patients had partial return and 1 patient had complete return of vocal fold movement within 10 months. Of the remaining 13 patients, 8 underwent vocal fold medialization with improvement of symptoms. Two patients are 6 and 7 months postinjury and await vocal fold medialization. Two patients are 27 months and 45 months postinjury and are considering vocal fold medialization. The remaining patient was lost to follow-up. An anatomic-geometric analysis of the right and left recurrent laryngeal nerves was performed by using measurements obtained from computed tomography scans of 8 patients with idiopathic unilateral VFP, as well as experience gained through surgical and cadaveric dissections. We conclude 1) the anterior cervical approach may place multiple branches of the vagus nerve at risk; 2) because of anatomic-geometric factors, the right-sided anterior cervical approach may carry a greater risk to the ipsilateral recurrent laryngeal nerve than does the left; and 3) an understanding of the anatomy and geometry presented herein allows relatively safe exposure from either side of the neck.

KEY WORDS — anterior cervical approach, cervical spine, complications, vocal fold paralysis.

INTRODUCTION

The anterior cervical approach is widely used for surgical access to the cervical spine.[1-5] Vocal fold paralysis (VFP), recognized as one of several potential complications of this procedure,[6,7] has not been sufficiently examined in the literature. Over the past 5 years we have treated 16 patients with VFP resulting from anterior cervical spine surgery. These 16 patients represent 6% of the 289 patients treated for VFP over this time period. As we gained experience we noted a marked preponderance of right-sided paralysis. The most obvious explanation for the right-sided preponderance was the anatomic differences between the left and right recurrent laryngeal nerves (RLNs). The most noteworthy of these differences are 1) the shorter loop of the right RLN and 2) the more lateral position of the inferior proximal right RLN as it passes under the subclavian artery (Fig 1). These differences were observed in oncologic surgery during cervical dissections that exposed the entire nerve segment, as well as in cadaver dissections. It appears that these anatomic differences may predispose the right RLN to a greater chance of injury with the retraction necessary to expose the anterior cervical vertebrae. Although the right side is more frequently used by most spine surgeons, this probably does not fully explain the marked preponderance of right-sided paralysis (94%) over left (6%).

We undertook further examination of this group to identify possible causes of this sidedness. Our goal was to offer new insights and recommendations regarding the side of approach used for the anterior access to the vertebral bodies.

MATERIALS AND METHODS

The database of patients with unilateral VFP seen in the Department of Otolaryngology–Head and Neck Surgery at Vanderbilt University Medical Center contains information on 289 such patients. Of these, 16 were found to have VFP following an anterior cervical approach for disc removal and fusion. The charts of these patients were reviewed and a more extensive database was completed for each. Opera-

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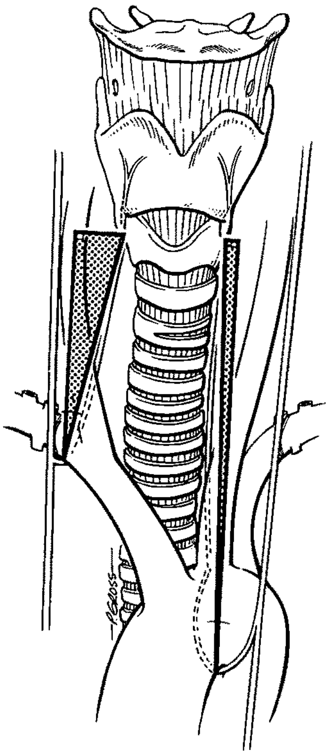

Fig 1. Anatomic drawing of each recurrent laryngeal nerve (RLN). Superimposed right triangles resolve course of each RLN into vertical and horizontal components. Right RLN has shorter, more oblique course than left RLN.

tive notes from the original procedures were obtained and reviewed. When necessary, follow-up phone calls were made to patients and referring surgeons in order to complete the database. The databases are maintained on a personal computer (Apple MacIntosh) using appropriate software (Filemaker Pro, Claris, Inc).

The relevant anatomy of the RLNs and adjacent structures were examined in anatomic texts, in cadaver dissections, and during cervical oncologic procedures in which the nerves were widely exposed. We also participated in two anterior cervical fusions to directly view the position of the Cloward retractor and the retraction or displacement of the cervical structures. The CT scans of 8 patients with idiopathic VFP (4 women and 4 men) were then reviewed to measure the vertical rise of the RLN on the left and right sides. The inferior edge of the cricoid cartilage, the innominate artery, and the inferior aspect of the aortic arch were identified, and the numbers of computed tomography sections between the landmarks were counted. Multiplying the number of sections by the section thickness (4 mm) yields an estimate of the vertical distances between the landmarks. The vertical distance from the innominate artery to the cricoid estimates the vertical rise of the right RLN, and the vertical distance from the inferior aspect of the aortic arch to the cricoid estimates the vertical rise of the left RLN.

Using the anatomic data from the various sources, we derived a geometric model to demonstrate the change in tension of the left and right RLNs produced during anterior cervical exposure.

RESULTS

The 16 patients with unilateral VFP following an anterior cervical approach were seen from 1990 to 1995. There were 9 women and 7 men ranging in age from 26 to 63 years. Fifteen had a right VFP and 1 had a left-sided paralysis. Twelve patients had a single fusion, 3 had fusion of two interspaces, and 1 had a fusion of three interspaces. Fourteen patients had a fusion of the C6-7 interspace, 6 had a C5-6 fusion,

TABLE 1. VOCAL FOLD PARALYSIS FOLLOWING ANTERIOR CERVICAL EXPOSURE IN 16 PATIENTS

Patient	*Age*	*Sex*	*Side*	*Disc Level*	*Hoarse*	*Breathy*	*Cough*	*Aspiration*	*Mass*	*Dysphagia*	*Vocal Fatigue*
1	36	F	L	C6-7	Y	N	Y	Y	N	N	Y
2	63	F	R	C6-7	Y	N	N	Y	Y	Y	Y
3	37	F	R	C6-7	N	Y	N	Y	N	N	Y
4	26	F	R	C6-7	N	Y	N	N	N	N	N
5	36	M	R	C6-7	Y	N	N	Y	N	Y	N
6	50	M	R	C5-6	Y	N	N	N	N	Y	Y
7	39	F	R	C4-5, C5-6, C6-7	Y	N	Y	N	N	Y	N
8	53	M	R	C6-7	Y	N	N	N	N	Y	N
9	50	M	R	C6-7	Y	N	N	N	N	Y	N
10	45	M	R	C6-7	Y	N	N	N	N	N	N
11	58	F	R	C5-6, C6-7	Y	N	N	Y	N	N	N
12	60	F	R	C5-6, C6-7	Y	N	N	N	N	N	Y
13	36	M	R	C6-7	Y	Y	N	Y	N	Y	N
14	41	M	R	C5-6, C6-7	Y	Y	N	N	N	N	N
15	37	F	R	C5-6	N	Y	N	Y	N	N	Y
16	43	F	R	C6-7	Y	Y	N	N	Y	Y	Y

TABLE 2. PRESENTING SYMPTOMS OF VOCAL FOLD PARALYSIS

	Transcervical (n = 16)		*Thyroidectomy (n = 22)*		*Endarterectomy (n = 13)*	
Symptom	*No.*	*%*	*No.*	*%*	*No.*	*%*
Hoarseness	13	81	14	64	10	77
Breathiness	6	38	14	64	5	38
Cough	2	13	0	0	2	15
Aspiration	7	44	6	27	4	31
Mass sensation	2	13	1	5	1	8
Dysphagia	8	50	6	27	4	31
Vocal fatigue	7	44	10	45	5	38

and 1 had a C4-5 fusion. Two patients had a previous anterior cervical fusion prior to the second procedure that caused VFP. Data from individual patients are outlined in Table 1.

Table 2 compares presenting symptoms among patients with VFP from an anterior cervical approach with symptoms from VFP resulting from thyroidectomy and carotid endarterectomy. The VFP from an anterior cervical approach has a higher incidence of dysphagia and aspiration than VFP resulting from thyroidectomy or carotid endarterectomy.

Treatment and Outcome. Four patients had partial or complete return of vocal fold movement within 10 months postinjury with improvement or resolution of symptoms. Of these, 1 had complete return of movement 1 month postinjury and 2 had partial return at 9 and 10 months postinjury. The third patient was lost to follow-up but by telephone reported no further voice or swallowing problems after a Gelfoam injection 1 month postinjury.

Of the remaining 12 patients, 8 underwent vocal fold medialization with improvement of symptoms. Two patients are 6 and 7 months postinjury and await vocal fold medialization if spontaneous resolution does not occur and symptoms persist. The remaining 2 patients are 27 months and 45 months postinjury and are considering vocal fold medialization.

Recurrent Laryngeal Nerve Measurements. Table 3 outlines the measurements obtained from the computed tomography scans of 8 patients with idiopathic VFP. The average (mean) vertical rise of the left RLN was 9.2 cm, and that of the right was 4.9 cm. Thus, the left RLN is 4.3 cm longer than the right RLN, or nearly twice as long ($p < .0001$, paired, two-tailed *t*-test). The last two columns of Table 3 will be addressed in Discussion.

DISCUSSION

Since its introduction in 1957,[2] the anterior approach for cervical fusion has become widely accepted and is described in neurosurgical and orthopedic textbooks[1-5] and journal articles.[6-8] The literature recognizes several potential complications of this procedure, including Horner's syndrome, esophageal perforation, vertebral artery injury, RLN injury, and cervical nerve root injury.[1] There exists considerable diversity among various authors regarding the cause and prevention of RLN injury during anterior cervical surgery. This diversity includes different opinions regarding the recommended side to use for the anterior approach. Bulger et al[6] advocate a right-sided approach, stating that a right RLN in its normal position is protected beneath the thyroid gland. However, they acknowledge that an aberrant, nonrecurrent nerve would be at risk for injury. Kelley and Whitecloud[1] state that while a right-sided approach may place the RLN at risk, a left-sided approach may place the thoracic duct at risk. They instruct the surgeon to use the side that is the most comfortable. Hoppenfield and deBoer[4] believe that while the side

TABLE 3. RECURRENT LARYNGEAL NERVE LENGTHS AND RELATIVE STRAIN

	RLN Rise (cm)		*Rise Difference*	*Ratio*	*Calculated % Strain*	
Patient	*Left*	*Right*	*L – R (cm)*	*L/R*	*Left*	*Right*
1	8.0	5.6	2.4	1.4	6.0	11.7
2	9.6	4.8	4.8	2.0	4.2	15.4
3	8.4	2.8	5.6	3.0	5.4	38.0
4	10.4	4.4	6.0	2.4	3.6	18.0
5	10.0	5.2	4.8	1.9	3.9	13.4
6	10.0	5.6	4.4	1.8	3.9	11.7
7	8.0	5.2	2.8	1.5	6.0	13.4
8	9.5	6.3	3.2	1.5	4.3	9.4
Mean	9.2	4.9	4.3	1.9	4.7	16.4
Median	9.6	5.2	4.6	1.9	4.7	13.3

Neck and chest computed tomography scans of 8 patients with idiopathic unilateral vocal fold paralysis were analyzed to estimate vertical rise of each recurrent laryngeal nerve (RLN). Last two columns show calculated percentage of strain placed on left and right RLNs by 2-cm horizontal displacement of larynx to contralateral side, as would occur during anterior cervical exposure.

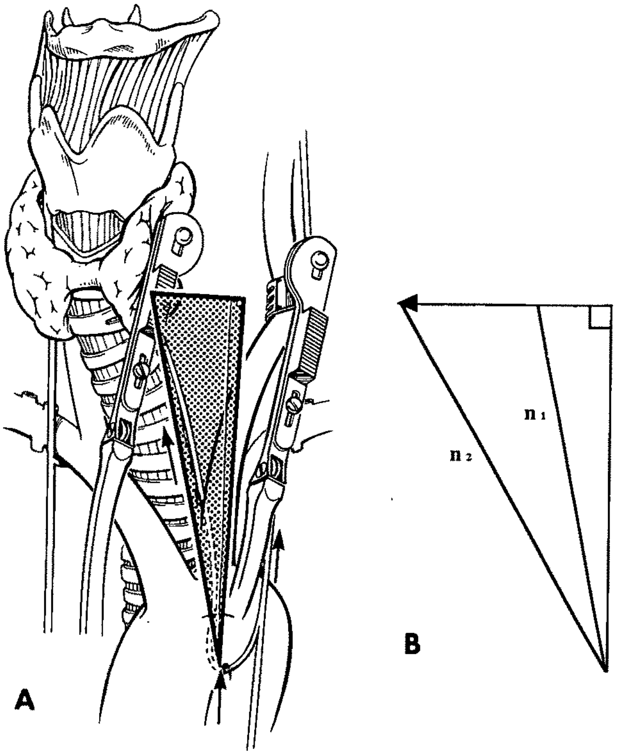

Fig 2. A) Anatomy and B) geometry of left RLN during anterior cervical exposure. Because of longer, more vertical course of left RLN, horizontal displacement of larynx to right by Cloward retractor stretches left RLN only slightly.

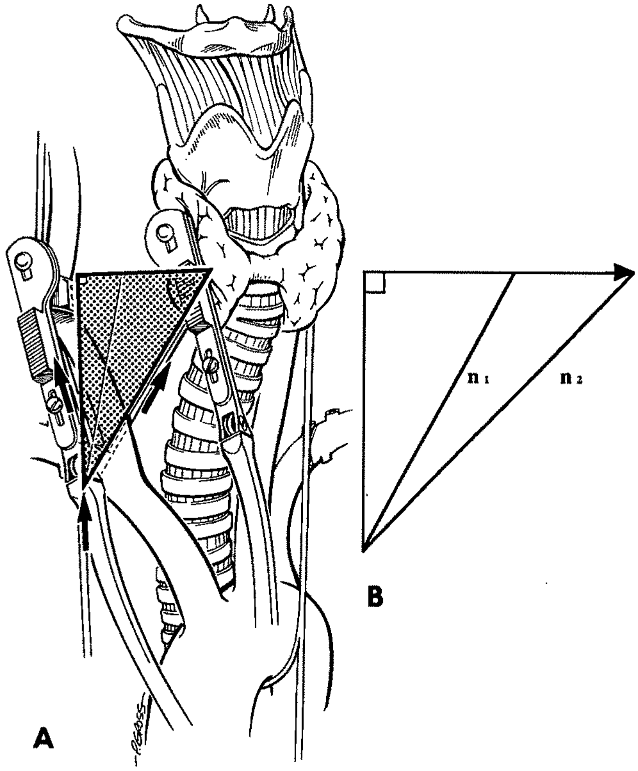

Fig 3. A) Anatomy and B) geometry of right RLN during anterior cervical exposure. Because of shorter, more oblique course of right RLN, horizontal displacement of larynx to left by Cloward retractor stretches right RLN significantly.

of the approach is the choice of the surgeon, a left-sided approach may be preferred because of the possibility of a nonrecurrent right inferior laryngeal nerve.

Two reviews specifically address the issue of RLN injury from an anterior cervical approach. In a review based on retrospective interviews and examinations of 102 patients, Bulger et al[6] noted a 22% incidence of temporary postoperative hoarseness, a 17% incidence of dysphagia, and a 1% incidence of permanent VFP. A right-sided approach was used in all cases. Heeneman[7] reviewed 85 patients and identified 9 cases (11%) with postoperative RLN injury. All but 6 were approached from the right side. The author outlines several possible mechanisms for RLN injury, including stretch of the nerve. He suggests that the shorter course of the right RLN places it at greater risk of stretch injury.

In our series, all but 1 patient (15 of 16) had a right-sided VFP, including one case of right VFP in which the operative note describes a left-sided approach. The two most likely causes for this right-sided preponderance are that 1) the right-sided approach is much more widely used than the left, and 2) the right-sided approach places the ipsilateral RLN at greater risk for injury than does the left-sided approach. These patients were acquired through a large referral network covering multiple states and referral centers. Moreover, because surgery on these patients was performed by a number of different surgeons, who in turn may be referring patients to multiple otolaryngologists, we cannot objectively measure the incidence of RLN injury in this study. Those referring surgeons whom we successfully contacted reported that they either 1) always use the right-sided approach or 2) use the most convenient side, depending on the side of the greatest disease or the dominant hand of the surgeon. Most do the former.

After reviewing the anatomy and geometry of the RLNs, we believe the right-sided approach carries a greater risk of injury to the RLN than does the left, even when the anatomy is normal. Figure 1 outlines the anatomy of the right and left RLNs. The right triangles superimposed on each side resolve the course of each RLN into horizontal and vertical components. The right RLN travels from its point of fixation near the thoracic inlet (the innominate artery) along an oblique course to its point of fixation at the larynx (the cricothyroid joint). The left RLN travels from its point of fixation in the chest (the inferior aspect of the aortic arch) on a longer, more vertical path to its point of fixation at the larynx (the cricothyroid joint). The

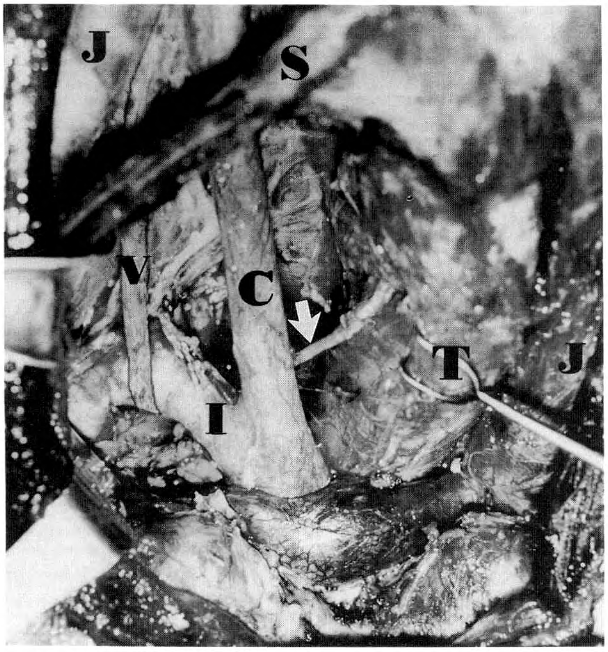

Fig 4. Intraoperative photograph illustrating stretch of right RLN by retraction of larynx to left. Thyroidectomy and paratracheal node dissection has been performed for neoplasm. Image has been digitally enhanced to improve clarity. J — internal jugular veins, S — strap muscle (sternohyoid), C — common carotid artery, I — innominate artery, V — vagus nerve, T — trachea, arrow — stretched RLN.

right RLN has a shorter vertical rise, a more oblique course, and a shorter overall length than the left RLN. These differences may render the right RLN more vulnerable to injury during anterior cervical exposure.

Figure 2 describes the anatomy and geometry of the left RLN during anterior cervical exposure. To expose the vertebral bodies, the Cloward retractor displaces the larynx horizontally to the right, stretching the left RLN slightly (Fig 2A). Figure 2B shows the same right triangle without the anatomic landmarks. When the larynx is horizontally displaced, the left RLN is stretched from length $n1$ to $n2$.

Figure 3 describes the anatomy and geometry of the right RLN during the anterior cervical approach. To expose the vertebral bodies, the larynx is displaced horizontally to the left, stretching the right RLN significantly (Fig 3A). Figure 3B shows the geometry more clearly as the right RLN is stretched from length $n1$ to $n2$. The intraoperative photograph (Fig 4) also illustrates these relationships.

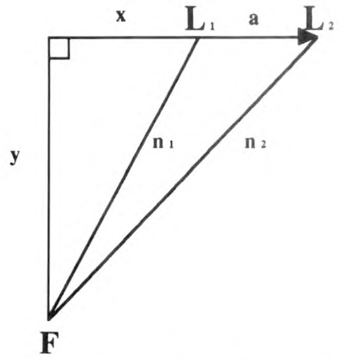

Fig 5. Geometric diagram of anterior cervical approach with horizontal displacement of larynx and stretch of RLN. See text for explanation.

The geometric differences between the right and left RLNs with respect to the anterior cervical approach may be mathematically defined (Fig 5). The terms x and y denote the horizontal and vertical distances from the point of fixation of the RLN in the chest F to its point of fixation at the larynx L1. The term $n1$ denotes the length of the RLN from F to L1. To expose the vertebral bodies, the larynx is displaced horizontally. This moves the laryngeal point of fixation from its normal location L1 to location L2. The RLN is thus stretched from resting length $n1$ to length $n2$. The term $n2/n1$ describes the proportional stretch of the nerve. Using the Pythagorean theorem of right triangles, the relationship of $n2$ to $n1$ can be described by the equation

$$\frac{n2}{n1} = \sqrt{1 + \frac{2ax + a2}{x2 + y2}}$$

and by definition, % strain = $100(n2/n1 - 1)$.

Study of this relationship shows that as y increases, $n2/n1$ approaches 1, or 0% strain. Conversely, as y decreases, strain increases for a given x and a. Because the right RLN has a shorter vertical course in the chest than the left RLN, the right RLN undergoes a greater degree of strain when the larynx is horizontally displaced to the contralateral side, as occurs in the anterior cervical approach.

Calculating the strain applied to the left and right RLNs by using estimated values for x and a shows the degree of stretch that may occur when the larynx is horizontally displaced. Figure 6 shows the relationship between the vertical rise (y) of the recurrent nerve and the degree of stretch. Assuming a horizontal distance (x) of 1 cm from the point of fixation in the chest to the cricothyroid joint for both right and left RLNs, and assuming that a horizontal laryngeal displacement (a) of 2 cm is required to expose the vertebral bodies, the degree of stretch exceeds 10% and rises rapidly when the vertical rise in the RLN is less than 6 cm. The last two columns of Table 3 show the calculated degree of strain that would occur during left- and right-sided anterior cervical approaches for each of the patients described. The average degree of strain for the right side is over three times that of the left.

With placement of the Cloward retractor to expose

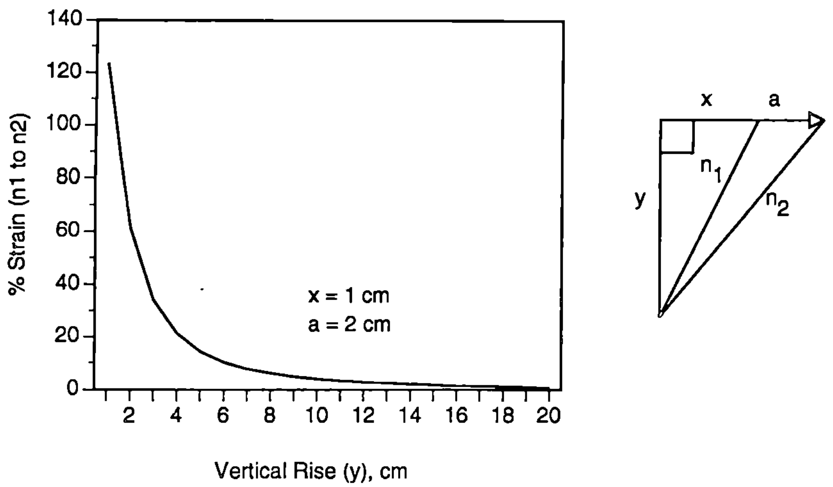

Fig 6. Relationship between vertical rise of RLN and stretch or strain applied to nerve by horizontal displacement of larynx during anterior cervical approach. Assumed, sample values for horizontal course of RLN (x = 1 cm) and horizontal displacement of larynx (a = 2 cm) are used. When vertical rise of RLN is less than 6 cm, stretch or strain applied to RLN with horizontal displacement of larynx exceeds 10%.

the anterior cervical vertebrae, the vagus nerve must be displaced laterally as the RLN is displaced medially. One can apply the same geometric model to the lateral displacement of the vagus nerve. The increased tension that is created within the vagus adds further tension to the RLNs as they pass under the subclavian artery or the aorta.

The orthopedic literature contains relevant data that address the relationship between strain and function in peripheral nerves. Studies of rabbit tibial nerve[8] show that for a 1-hour trial, 6% stretch produced partial loss of function (electromyographic amplitude) that resolved completely with relaxation, and 12% strain caused complete loss of function with only partial recovery following relaxation. Studies of rabbit sciatic nerve[9] show complete cessation of blood flow at 16% strain.

During anterior cervical exposure, the RLN is swept medially with the tracheoesophageal unit without direct visualization of the nerve during the approach. Because of the more lateral course of the right RLN, it is also at greater risk of direct trauma in the more proximal segment of the nerve, especially during surgery of the more inferior levels. Figure 1 illustrates how the left RLN maintains close proximity to the tracheoesophageal groove while the right RLN, because of its sling under the subclavian artery, courses more laterally in the peritracheal space prior to reaching the protection of the tracheoesophageal groove and thyroid gland. The risks of direct trauma may increase in revision surgery, in which the position of the RLN may be altered by scarring from the previous procedure.

It is wise to be aware of the possibility of a nonrecurrent inferior laryngeal nerve during any right lower cervical surgical procedure. However, this anomaly is so rare it probably plays a very minor role in the sidedness of injury of the RLN during anterior cervical surgery.

Our analysis suggests that stretch or strain plays a significant role in the mechanism of RLN injury during the anterior cervical approach. Horizontal displacement of the larynx to expose the cervical spine can stretch the RLN to a degree that has been demonstrated experimentally to cause injury in peripheral nerves. Furthermore, because of differences in vertical rise between the right and left RLNs, the right RLN appears more vulnerable to such an injury than the left RLN. Furthermore, if the Cloward retractor is positioned inferior to the cricoid cartilage, the nerve is trapped in a more inferior position, increasing the tension even further.

We have also observed that compared to VFP following thyroidectomy or carotid endarterectomy, VFP following anterior cervical exposure has a higher incidence of dysphagia and aspiration. The small number of patients in this series prevents statistical analysis of this observation. However, we believe that horizontal displacement of the larynx may also stretch the superior laryngeal and pharyngeal branches of the vagus nerve. This may help explain the increased incidence of dysphagia and aspiration seen in this series. For these branches, the risk of injury for the right and left sides is probably similar.

The literature currently lacks a consensus regarding the preferred side for the anterior cervical approach. We suggest that the left side be given greater consideration for this approach in order to minimize the risk of RLN injury. The shorter, more lateral position of the right RLN exposes it to an increased risk of injury from both direct trauma and stretch injury. Dissection in the left side of the neck medial to the carotid artery and internal jugular vein carries minimal risk of thoracic duct injury. Furthermore, the right side of the neck may also contain large lymphatic channels that have a similar (but low) risk of injury from a right-sided approach.

The major drawback to this study is the lack of availability of data from the referring institutions regarding the total number of anterior cervical approaches performed within the same time period and the incidence of right versus left approaches. If 94% of the procedures were performed on the right, then the majority of right-sided cases merely reflects this percentage. However, the strong implication of these data is that there is more risk during the right-sided approach. Another limitation is the lack of direct in vivo measurements of nerve length and tension changes during laryngeal displacement. It would be difficult to obtain measurements during anterior cervical surgery without increasing the risk of nerve injury. We are presently designing studies that address these issues.

CONCLUSIONS

In reviewing our series of 16 patients with VFP following an anterior cervical approach, we have observed a preponderance of right-sided occurrences. Although we acknowledge that most neurosurgeons and orthopedic surgeons use the right-sided approach and that permanent, symptomatic paralysis following the procedure is uncommon, we suggest that the incidence of temporary, partial, or asymptomatic paralysis may be more frequent and go relatively unnoticed. More frequent use of the left-sided approach may reduce the risk of such injuries. Regardless of the side used, the risk of injury to the pharyngeal and superior laryngeal branches of the vagus nerve also exists.

An understanding of the anatomic and geometric relationships outlined in this paper will benefit neurosurgeons, orthopedic surgeons, and otolaryngologist–head and neck surgeons who operate on or near the RLN. Although the right side may carry a greater risk of injury to the nerve, with careful consideration of the above anatomic differences, surgery can be performed relatively safely on either side.

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PEDIATRIC OTORHINOLARYNGOLOGY ENDOSCOPY DAYS

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SUPRAGLOTTIC AND PHARYNGEAL SENSORY ABNORMALITIES IN STROKE PATIENTS WITH DYSPHAGIA

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Dysphagia and aspiration are two devastating sequelae of stroke, accounting for nearly 40,000 deaths from aspiration pneumonia each year in the United States. While motor deficits in the larynx and pharynx are thought responsible for dysphagia and aspiration in stroke patients, no prior study has evaluated whether these patients also have sensory deficits. The aim of this study was to evaluate the sensory capacity of the laryngopharynx (LP) in supratentorial or brain stem stroke patients who presented with dysphagia. Fifteen stroke patients (mean age, 66.7 ± 13.8 [SD] years) were prospectively evaluated by means of our previously described method whereby air pulse stimuli were delivered via a flexible fiberoptic telescope to the mucosa innervated by the superior laryngeal nerve. There were 15 age-matched controls. No LP sensory deficits were found in any of the age-matched controls. In all stroke patients studied, either unilateral ($n = 9$) or bilateral ($n = 6$) sensory deficits were identified. Deficits were defined as either a moderate impairment in sensory discrimination thresholds (3.5 to 6.0 mm Hg) or a severe sensory impairment (>6.0 mm Hg). These sensory discrimination thresholds were significantly greater than in age-matched controls (7.05 ± 0.17 mm Hg for the supratentorial group and 6.05 ± 1.22 mm Hg for the infratentorial group versus 2.61 ± 0.69 mm Hg for the controls). Among patients with unilateral deficits, sensory thresholds were moderately to severely elevated in all 9 cases on the affected side compared with the unaffected side ($p < .01$, Fisher's exact test). Moreover, the sensory thresholds of the unaffected side were not significantly different from those of age-matched controls (2.51 ± 0.25 mm Hg versus 2.61 ± 0.69 mm Hg, respectively). All 6 patients with bilateral deficits had severe impairments. The results of an outcome assessment in 13 of 15 patients revealed that 2 out of 5 patients with moderate LP sensory impairment and 5 out of 8 with severe impairment developed aspiration. Our results show for the first time that stroke patients with dysphagia have significant sensory deficits in the LP and that these impairments are likely to contribute to the development of aspiration.

KEY WORDS — aspiration, dysphagia, pharyngeal sensory testing, sensory impairment, stroke, swallowing.

INTRODUCTION

Stroke is the third most common cause of death in the United States, after cardiovascular disease and cancer, accounting for approximately 150,000 deaths a year.[1] Because stroke is more often disabling than fatal, it is a major public health problem, resulting in health care expenditures and lost productivity of greater than $19 billion in 1993.[2] Death following stroke is usually due to non-neurologic causes such as cardiovascular disease and pneumonia.[3] Studies examining the natural history of stroke have shown that there is a 20% incidence of death from aspiration pneumonia in the first year after a stroke, and that each year thereafter, 10% to 15% of deaths following stroke are due to aspiration pneumonia.[4] Extrapolating to the current population of the United States, aspiration pneumonia after stroke is responsible for approximately 40,000 deaths a year.

One of the most devastating and common complications of stroke is severe dysphagia, which occurs in roughly 30% of all stroke patients.[5] Dysphagia may lead to aspiration, pneumonia, and death.[6,7] Usually, it is not the first episode of aspiration pneumonia in victims of stroke that leads to demise. Rather, it is the subsequent episodes of recurrent aspiration pneumonia over a several-year period that eventually cause these large numbers of patients to succumb.[7,8] A combination of factors have been shown to be responsible for the dysphagia that often occurs in stroke patients, including absence or severe delay in the triggering of the swallowing reflex,[9] cricopharyngeal dysfunction,[10] reduced lingual control, reduced gag reflex, reduced strength of pharyngeal contraction, discoordinated peristalsis or dysmotility, and reduced

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strength of cough.[7] All of these are problems with motor function. While motor dysfunction clearly plays a role in dysphagia and aspiration in stroke patients, no prior study has examined whether these patients also have sensory deficits of the laryngopharynx (LP). Sensory dysfunction in this region would be expected to impair laryngeal reflexes and voluntary LP protective mechanisms. Until recently, however, it had not been possible to reliably measure sensory capacity in the LP. We have overcome this problem with the development of a new technique.[11] As previously described, our approach uses discrete air pulse stimulation of the mucosa innervated by the superior laryngeal nerve (SLN) in order to reliably determine sensory discrimination thresholds.[11,12]

It is well known that patients with stroke have both motor and sensory deficits affecting the limbs and trunk.[13] Defects in the ascending sensory pathways are readily documented by routine physical examination of the body surface. It is not likely that the pharynx and supraglottic larynx are spared following ascending sensory pathway lesions. The contribution of pharyngeal and supraglottic laryngeal sensory deficits toward the development of dysphagia and aspiration in stroke patients has never been determined. In this study, we began to answer this question by determining LP sensory capacity in stroke patients with dysphagia. We also assessed the relationship between LP sensory changes and clinical outcome.

MATERIALS AND METHODS

We prospectively studied 15 patients who suffered either a supratentorial or brain stem stroke and who either complained of, or were noted to have, dysphagia. Dysphagia was defined as any subjective or objective difficulty in swallowing solid or liquid food, or coughing or choking while swallowing. Nine men and 6 women with a mean age of 66.7 ± 13.8 years were studied. There were 15 age-matched controls. Ten patients had infratentorial strokes; 8, strokes involving the lateral medulla; and 2, strokes involving other brain stem sites. Eight of the infratentorial stroke patients had unilateral strokes, and 2 had bilateral strokes. Five patients had supratentorial strokes, 2 of whom had bilateral strokes. All patients were studied within 4 weeks of the onset of their stroke.

Psychophysical testing was used to determine sensory discrimination thresholds in the supraglottic larynx and pharynx. The patients were evaluated by our previously described method whereby air pulse stimuli were delivered via a flexible fiberoptic telescope to the mucosa innervated by the SLN.[11,12] Briefly, a pressure- and duration-controlled puff of air was delivered to the anterior wall of the pyriform sinus (the area innervated by the SLN). The air puff was obtained from the standard air pump found in most otolaryngology offices for the delivery of aerosolized medications (SMR Maxi Cabinets, Storz Surgical Specialties, St Louis, Mo). The air puff was ejected from an internal port located within a flexible fiberoptic laryngoscope made especially for this purpose (Pentax Precision Instrument Corporation, Orangeburg, NY). To determine an individual's sensory pressure threshold, air pressure was varied according to the psychophysical method of limits while the duration of the air puff was held constant at 50 milliseconds.

Prior to sensory testing, the patient was provided a detailed description of the apparatus and procedure, then was given the opportunity to feel an air puff on his or her cheek and comment on the sensation. The floor of the nose was then anesthetized with 1% Xylocaine with epinephrine 1:100,000 placed on a cotton-tipped applicator and inserted 1 cm from the nasal vestibule. No other anesthesia was given. Next, the flexible laryngoscope was inserted into the nasal passage and the patient was given a 1-minute rest period to adapt to the laryngoscope and prepare for testing.

Testing began by orienting the subject to the supraglottic stimulus with a suprathreshold stream of air for 5 seconds. After a 15-second rest period we began presenting air puffs. Six blocks of stimulus administration trials were given in which a threshold was obtained for each block. One stimulus was presented per trial. The trial duration was approximately 10 seconds with a 10-second intertrial interval. After a verbal announcement of the beginning of the trial, the air puff was administered at a random time from 2 to 8 seconds within the 10-second trial interval. Three blocks of descending pressure stimulus presentations and three blocks of ascending pressure stimulus presentations were randomly ordered for each subject. Within a block, air puffs were presented in sequential steps of 7.5×10^{-3} mm Hg: from subliminal to supraliminal for ascending blocks, and from supraliminal to subliminal for descending blocks. Subjects reported detection of the stimulus by raising their hand within 2 seconds of stimulus presentation. A 30-second rest period separated the blocks. The mean of the lowest detected pressures from the six blocks was used as that subject's sensory threshold.

Two modifications to the ascending-descending method of limits were introduced to ensure accurate threshold determinations. First, we introduced catch trials in which a stimulus was not presented. This was

TABLE 1. STROKE DATA

Demographic information	
Name: ______________	
MRN: __________	
Sex: ____ 0 = F 1 = M	
Age: ____	
Race/ethnicity: ____	0 = white, NH, 1 = black, NH, 2 = Hispanic, 3 = other
Date of CVA: ______	
Stroke type: ______	
Examination features	
Dysphagia: ____	0 = none, 1 = mild, 2 = moderate to severe
Dysarthria: ____	0 = none, 1 = mild, 2 = moderate to severe
Palatal weakness: ____	0 = none, 1 = mild, 2 = moderate to severe
Gag impairment: ____	0 = none, 1 = mild, 2 = moderate to severe
Facial sensory loss: ____	0 = none, 1 = mild, 2 = moderate to severe
Facial motor loss: ____	0 = none, 1 = mild, 2 = moderate to severe
Body sensory loss: ____	0 = none, 1 = mild, 2 = moderate to severe
Body motor loss: ____	0 = none, 1 = mild, 2 = moderate to severe
CT/MRI information	
Scan type: CT MRI	
Location of CVA: ______________________	
Brain stem?: ____	0 = no, 1 = yes
Side affected: ____	0 = L, 1 = R, 2 = both
Size of stroke: ____	0 = small, 1 = medium, 2 = large
Old strokes?: ____	0 = no, 1 = yes
Outcome	
Aspiration: ____	0 = no, 1 = yes
PEG: ____	0 = no, 1 = yes
Death: ____	0 = no, 1 = yes

MRN — Medical Record Number, NH — non-Hispanic, CVA — cerebrovascular accident, PEG — percutaneous endoscopic gastrostomy.

accomplished by reducing stimulus intensity to zero but continuing to trigger solenoid valve closure. The absence of a patient's response on these trials verified that he or she was responding to the air puff stimulus and not to an extraneous cue such as the masking sound, solenoid click, etc. This also confirmed that the test subject's level of cognition was such that he or she was able to successfully undergo this psychophysical test. Second, we presented stimuli using a single-blind procedure in which the examiner did not have knowledge of stimulus intensity. This was accomplished by having an associate working with the examiner set the stimulus intensity. In this way, the examiner did not know the starting subliminal stimulus intensity, the intensity of the largest supraliminal stimulus used, or on which trials the stimulus was not presented (catch trials). Moreover, the examiner did not know if the stimulation block consisted of air puffs presented in ascending or descending order.

Both the right and left sides of the pharynx and supraglottic larynx were studied. The duration of the examination was, on average, no more than 10 minutes longer than the time it takes to perform a typical flexible fiberoptic laryngoscopic examination.

In addition to LP sensory testing, we assessed up to eight of the following physical examination features: severity of dysphagia, dysarthria, palatal weakness, gag impairment, facial motor and sensory loss, and body motor and sensory loss. Dysphagia, dysarthria, palatal weakness, and gag impairment were assessed by a rating scale ranging from 0 to 2, where 0 was rated as no deficit, 1 a mild deficit, and 2 a moderate to severe deficit. The presence or absence of motor control and facial and body sensation to touch was also noted. To prevent bias, the sensory testers (J.E.A., M.S.K., A.B.) were blinded to the severity of dysphagia prior to testing.

TABLE 2. LARYNGOPHARYNGEAL SENSORY THRESHOLD

	Mean ± SD (mm Hg)
Supratentorial (n = 5)	
3 Unilateral deficits	7.00 ± 0.22
2 Bilateral deficits	7.12 ± 0.00
Total	7.05 ± 0.17
Infratentorial (n = 10)	
6 Unilateral deficits	5.65 ± 1.36
4 Bilateral deficits	6.84 ± 0.51
Total	6.05 ± 1.22

TABLE 3. LARYNGOPHARYNGEAL SENSORY DEFICIT AND SEVERITY OF DYSPHAGIA

	Dysphagia		
Laryngopharyngeal Sensory Deficit	*Mild*	*Moderate or Severe*	*Total*
Moderate	2	3	5
Severe	3	7	10
Total	5	10	15
Side			
Left	4	3	7
Right	0	2	2
Both	0	6	6
Total	4	11	15

Outcome was assessed in two ways: 1) by determining if the patients in the study aspirated or died prior to discharge from the hospital and 2) by determining if the patient required a percutaneous gastrostomy tube for alimentation. The data collection form used for each patient is shown in Table 1.

STATISTICAL ANALYSIS

Fisher's exact test was used to test differences between the affected side of the pharynx and the unaffected side. Fisher's exact test also was used to test differences between stroke patients and healthy controls.

RESULTS

All of the test subjects successfully completed the sensory testing procedure without responding to a catch trial. Furthermore, for each individual patient studied, sensory thresholds did not vary more than 0.50 mm Hg from their particular sensory pressure threshold, and the six block trial thresholds were very consistent.

In all patients studied, either unilateral (n = 9) or bilateral (n = 6) LP sensory deficits were identified. No LP sensory deficits were found in any of the age-matched controls. Deficits were defined as either a moderate impairment in sensory discrimination thresholds (3.5 to 6.0 mm Hg), seen in 4 patients (all infratentorial strokes), or a severe sensory impairment (>6.0 mm Hg), seen in 11 patients. The elevations in sensory discrimination thresholds were significantly greater than those in age-matched controls (7.05 ± 0.17 mm Hg for the supratentorial group and 6.05 ± 1.22 mm Hg for the infratentorial group versus 2.61 ± 0.69 mm Hg for the controls; Table 2).

Of the 5 patients with supratentorial strokes, 3 had unilateral infarcts. The LP sensory deficits that we identified in these patients were located contralateral to the side of the infarct. The 2 supratentorial stroke patients with bilateral lesions had bilateral LP sensory deficits. Six of the 10 infratentorial stroke patients had unilateral lesions, and the LP sensory deficits in this group were all ipsilateral to the affected brain stem sites. Four of these 6 patients, all with lateral medullary infarcts, had ipsilateral vocal cord paralysis. No other patients in our study had vocal cord paralysis. Patients with bilateral infratentorial lesions (n = 4) had bilateral LP sensory deficits.

Among the 9 patients with unilateral deficits, sensory thresholds were significantly elevated on the affected side compared with the unaffected side ($p < .01$, Fisher's exact test). All 9 of these patients had moderate to severe elevations in sensory threshold. Sensory thresholds of the unaffected side were not significantly different from those of age-matched controls[12] (2.50 ± 0.24 mm Hg versus 2.44 ± 0.19 mm Hg, respectively). All 6 patients with bilateral deficits had severe impairments.

In two thirds of the cases, the clinical assessment of severity of dysphagia by the neurologist (the results of which were unknown to the sensory testers) correlated well with the severity of LP sensory deficit. In one third of the cases, however, the clinical

TABLE 4. LARYNGOPHARYNGEAL SENSORY DEFICIT AND SEVERITY OF GAG IMPAIRMENT, PALATAL WEAKNESS, AND DYSARTHRIA

	Gag Impairment				*Palatal Weakness*				*Dysarthria*			
LP Sensory Deficit	*None*	*Mild*	*Moderate to Severe*	*Total*	*None*	*Mild*	*Moderate to Severe*	*Total*	*None*	*Mild*	*Moderate to Severe*	*Total*
Moderate	0	3	2	5	0	4	0	4	1	3	1	5
Severe	1	7	2	10	1	5	2	8	2	1	6	9
Total	1	10	4	15	1	9	2	12	3	4	7	14

TABLE 5. LARYNGOPHARYNGEAL SENSORY DEFICIT AND SENSORY AND MOTOR LOSS

LP Sensory Deficit	*Facial Sensory*			*Body Sensory*			*Facial Motor*			*Body Motor*		
	No	*Yes*	*Total*	*No*	*Yes*	*Total*	*No*	*Yes*	*Total*	*No*	*Yes*	*Total*
Moderate	0	2	2	0	4	4	2	3	5	2	2	4
Severe	3	4	7	3	5	8	1	8	9	1	7	8
Total	3	6	9	3	9	12	3	11	14	3	9	12

assessment of dysphagia either overestimated or underestimated the severity of the LP sensory deficit as judged by the sensory test (Table 3).

We observed that all patients experienced, to various degrees, dysarthria, palatal weakness, and gag impairments. While there was no reliable association between palatal weakness or gag impairment and level of LP sensory deficit, there was an association between dysarthria and LP sensory deficit (Table 4).

In patients in whom body and facial motor and sensory testing could be completed, there was no discernable association between level of LP sensory deficit and facial or body motor and sensory loss (Table 5).

We obtained outcome data on 13 patients. Two of 5 patients with moderate LP sensory deficits were found to aspirate, while 5 of 8 patients with severe LP sensory deficits aspirated, 1 of whom died. A percutaneous gastrostomy tube was required in 2 of 5 patients with moderate LP sensory deficits, and in 4 of 8 patients with severe LP sensory deficits (Table 6).

DISCUSSION

This current study demonstrates, for the first time, that stroke patients with dysphagia have significant sensory deficits in the LP. Clinical assessment of severity of dysphagia accurately predicted severity of LP sensory deficits in only two thirds of the cases. In general, the management of stroke patients with dysphagia is complicated by the fact that there is no reliable guide as to when a patient's diet should be modified, or when a patient should be alimented other than by mouth. The potential health risk to the patient with dysphagia and aspiration is great. Quantitative sensory evaluation could provide an objective criterion upon which the decision for a particular dietary change is based. Moreover, by attaching a number to the deficit, patients can better understand why a specific dietary change is prescribed.

TABLE 6. LARYNGOPHARYNGEAL SENSORY DEFICIT AND OUTCOME

LP Sensory Deficit	*Aspiration*			*PEG*		
	No	*Yes*	*Total*	*No*	*Yes*	*Total*
Moderate	3	2	5	3	2	5
Severe	3	5*	8	4	4	8
Total	6	7	13	7	6	13

*There was one death in this group of aspirating patients.

Our outcome data suggest that the severity of LP sensory deficits predicts predisposition to aspiration. In more subtle clinical situations, where evidence of dysphagia or aspiration might not be readily apparent, LP sensory testing would be critical. We hypothesize that an LP sensory deficit without clinical evidence of dysphagia (ie, silent sensory deficit) is insidiously dangerous to the patient, as it might predispose to developing silent aspiration.[14]

A modified barium swallow study is currently an essential part of the workup and management of stroke patients with dysphagia, yet the modified swallow study, in its general use, specifically visualizes only the motor component of swallowing, and only indirectly visualizes the sensory component (secondary reflex events and aspiration).[15]

Previous clinical studies attempting to examine the relationship between the site of a cerebrovascular accident, the nature of the dysphagia exhibited, and the frequency and cause of aspiration have been inconclusive.[16-18] These studies have shown that both right- and left-sided strokes may result in dysphagia and aspiration. None of these studies addressed the issue of supraglottic and pharyngeal sensory deficits as a contributing factor in the development of dysphagia and aspiration. It is likely that the sensory deficits we observed in stroke patients are related to disruption of an ascending projection pathway transmitting sensory information from the LP mucosa to the diencephalon and cortex. We suggest this because, in unilateral stroke patients, there was a striking correspondence between the side of the lesion and that of the LP sensory defect. For infratentorial strokes, LP sensory deficits were ipsilateral to the side of the stroke, while for supratentorial strokes, deficits were contralateral to the lesion. This is consistent with a predominantly crossed pathway, with a brain stem decussation rostral to the site of the lesion.

Because of the historical difficulty in assessing LP sensory discrimination, other physical examination methods have been used as a facsimile or next-best method to make this assessment.[13] Traditionally, assessment of the gag reflex has been used as a gross means of LP sensory assessment. While our patient population is small, there appears to be no reliable correlation between severity of LP sensory deficits and severity of gag impairment. For example, there were 5 patients with mild impairment in the gag reflex, but the LP sensory deficit was severe. This lack of correlation is not surprising, since the gag reflex measures afferent activity in the 9th cranial nerve, not the SLN branch of the 10th cranial nerve.

CONCLUSION

This study has demonstrated that a sensory deficit exists in the pharynx and supraglottic larynx in stroke patients with dysphagia. This sensory deficit is apt to play a significant role in the development of aspiration in these patients, both directly, by reduced stimulus detection and discriminative capacity in the LP, and indirectly, in impaired triggering of motor actions. Our provisional outcome data suggest that LP sensory deficits predispose stroke patients to aspiration. Thus, our results show that quantitative sensory evaluation of the supraglottic larynx and pharynx provides an objective and reproducible criterion for managing the rehabilitation of these patients.

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PATHOPHYSIOLOGY OF REACTIVE AIRWAY DISEASE AND SINUSITIS

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The association between asthma and sinusitis was recognized more than a century ago. Since 1980, several studies have documented that severe asthma improved after coexisting sinusitis was effectively treated either medically or surgically. Because the mechanism relating sinusitis to asthma is not known, several theories have been proposed: 1) aspiration of infected sinus secretions into the lungs during sleep, 2) enhanced vagal stimulation in the infected sinus producing direct bronchospasm, 3) bronchospasm from excessive airway drying from mouth breathing, 4) production of bacterial toxins that induce partial beta blockade, and 5) production in the infected sinus of cytokines and bronchoconstrictive mediators. There are data to support each of these hypotheses, and any or all of them may be operative. In view of recent demonstrations of activated lymphocytes and eosinophils in asthmatic airways, it is intriguing that biopsies of chronic hypertrophic sinusitis have revealed increased numbers of eosinophils and increased levels of granulocyte-macrophage colony stimulating factor, interleukin-3, and interleukin-5 compared to control tissue. These findings suggest that sinusitis might induce asthma by stimulating eosinophil production and activation and thereby supplying peptidoleukotrienes (LTC4 and LTD4) and other asthmagenic eosinophil products.

KEY WORDS — asthma, cytokine, eosinophil, granulocyte-macrophage colony stimulating factor, interleukin, lymphocyte, reactive airway disease, sinusitis.

The association between asthma and sinusitis was recognized more than a century ago. In the 1920s and 1930s, considerable attention was paid to the coincidence of sinusitis and asthma. This interest waned, and then in the past 20 years the subject has again commanded attention.[1] In 1969, Kaufman and Wright[2] exposed the mucosa of the nose and nasopharynx of 10 patients with no pulmonary disease to silica particles and observed significant increases in airway resistance as determined by body plethysmographic studies. This effect was abolished by atropine. Further evidence of the direct relationship between the upper and lower airways in response to immunologic stimuli was demonstrated by using inhalation challenges of ragweed antigen in patients with allergic rhinitis as well as patients with allergic asthma; the two groups of patients could not be distinguished on the basis of their lung responses.[3]

Rachelefsky et al[4] reported a group of 48 children with asthma and sinusitis in whom the asthma dramatically improved when the sinusitis was effectively treated with antibiotics and decongestants. Slavin[5] reported on a group of 33 adult patients with sinus disease and asthma, of whom 5 were not receiving corticosteroids, 10 were receiving frequent bursts, and 18 were on continuous steroids averaging 20 mg/d. All of these patients received intensive medical therapy including nasal decongestants and up to 1 month of an antibiotic. Two of these patients responded to medical therapy alone, 2 required the addition of intranasal antrostomy, 3 required bilateral Caldwell-Luc procedures, and 26 required bilateral intranasal sphenoethmoidectomy. Eighty-five percent of these patients noted subjective improvement in their asthma, and 15 of the 18 patients on continuous steroids reduced their doses from an average of 20 mg/d to an average of 3 mg/d. Thus, it has been well documented in both adults and children that effective control of sinusitis can result in marked improvement of asthma.[4-6]

The mechanism by which sinusitis might induce or aggravate asthma is not known. Theories that have been proposed include 1) aspiration of infected sinus secretions into the lower airways during sleep, 2) enhanced vagal stimulation in the infected sinus producing direct bronchospasm, 3) excessive drying of the lower airways by mouth breathing because of nasal obstruction, 4) production of bacterial toxins that induce partial beta blockade, and 5) production in the infected sinus of cytokines and bronchoconstrictive mediators. With regard to No. 1, above, it has been documented that nasopharyngeal secretions gain access to the lung in 50% of normal subjects and 70% of ill patients because of relaxation of the epiglottis during sleep[7]; however, as opposed to the purulent drainage from the sinuses themselves, the sputum of

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most asthmatics is sterile or contains only typical respiratory flora, and the chest radiograph of the typical asthmatic patient with sinusitis reveals no suggestion of pneumonia or pneumonitis. Although there may be some vagal stimulation from an infected sinus as suggested ın No. 2, above, the failure to control asthma in the face of sinusitus with anticholinergic therapy speaks against this theory as the primary explanation for the asthma. While there may be drying of the lower airways in cases of sinusitis with asthma, the addition of humidification renders only moderate symptomatic relief, so that No. 3, above, is certainly not a satisfactory explanation. Szentivanyi[8] demonstrated and developed considerable excitement about the partial beta blockade theory of asthma. Although asthmatics demonstrate some similarities to animals with experimentally produced beta blockade, no likely causative bacterial toxins have been demonstrated in either the upper or lower airways of patients with asthma, and the beta blockade theory fails to account for the recently appreciated inflammatory changes in the airways of asthmatics. This review will therefore focus on No. 5, reviewing the inflammatory aspects of sinusitis and asthma and the likely importance of cytokines and bronchoconstrictive mediators.

Bronchial biopsy and bronchoalveolar lavage in asthmatics reveal activated CD4+ T lymphocytes, activated eosinophils (EOSs), and neutrophils, in addition to the customary mast cells and alveolar macrophages. Mast cells activated by either the antigen-IgE pathway or soluble mediators such as formylmethionyl-leucyl-phenylalanine (fMLP) not only release histamine, a potent bronchoconstrictor, but also produce and release the more potent prostaglandins (PGs), particularly PGD2.[9-12]

The EOS is a bone marrow–derived cell that circulates and has a life span of approximately 3 days. It is programmed to undergo gradual disintegration, called apoptosis, through orderly destruction of the nucleus by endonucleases. The EOS is rapidly destroyed by the influence of adrenal corticosteroid. When the EOS encounters granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3), or interleukin-5 (IL-5), apoptosis is inhibited, the cell becomes steroid-resistant, and the EOS becomes activated: it becomes hypodense, it is primed for antibody-dependent cellular cytotoxicity, and it will release increased quantities of mediators in response to a challenge. The EOS very late acting (VLA-4) adhesion receptor is able to interact with the endothelial adhesion molecule known as vascular cell adhesion molecule (VCAM-1), to enter tissues such as the lung or the sinus.[12-14]

Mast cells, basophils, and EOSs are the only bone marrow–derived cells found in the lung that can convert their own arachidonic acid to the cysteinyl leukotriene LTC4, which is converted to LTD4 and then to LTE4.[12] These LTs, collectively constituting what was formerly called the slow-reacting substance of anaphylaxis (SRS-A), are 1,000 times as potent as bronchoconstrictors as is histamine.[15] The administration of highly effective LT inhibitors to asthmatics reduces airway narrowing, strongly suggesting that chronic LT production is at least partially responsible for asthmatic airway constriction. There is evidence, also, that LTE4 enhances airway hyperirritability.[12]

In a recent study of asthmatic patients admitted to a hospital for acute asthma, 85% of 110 patients had a radiographic abnormality of one or more paranasal sinuses, so that the sinus radiographs resulted in alteration of therapy in 29% of the patients.[16] In a different study of 104 patients undergoing endoscopic surgery for chronic sinusitis, extensive disease was present in 39% of the patients and correlated well with asthma and both tissue and peripheral EOSs.[17]

A recent report details the immunohistologic findings in chronic hyperplastic sinusitis.[18] Sinus biopsies were performed in 12 adult patients, of whom 6 were men and 6 women, 11 were asthmatic, and 5 were allergic. An EOS-predominant inflammatory infiltrate was seen in 10 of the 12 patients, mostly in diseased tissues. There was no difference between normal and diseased tissues in their content of CD3+ or CD4+ lymphocytes, elastase-positive neutrophils, or CD68+ macrophages. The sinusitis tissues showed significantly higher numbers of GM-CSF and IL-3 mRNA+ cells than normal control tissues. There was a significant correlation between the number of infiltrating activated EOSs and cells expressing mRNA for GM-CSF and IL-3. There was no correlation between IL-5 mRNA expression and infiltrating EOSs in diseased tissues. The IL-5 density was significantly higher in the tissues of the allergic patients than in the 7 control patients or normal control subjects.

Taken together, the above observations reveal a strong relationship between the presence of sinusitis and the presence of asthma. Further, when sinusitis is effectively treated, concurrent asthma is likely to be fully or better controlled.[4-6] Activated CD4+ T lymphocytes are present in both lung tissues of asthmatics and in diseased sinus tissues, as are activated EOSs.[18,19] Diseased sinus tissues contain mRNA for both GM-CSF and IL-3, and either of their products is capable of activating EOSs.[12,18] With the observation that peripheral eosinophilia correlates strongly with extensive sinus disease,[17] it is tempting to speculate that EOSs activated in sinusitis tissue find their way to the lung, where they contribute to airway narrowing by the production of leukotrienes as well as the release of EOS major basic protein and other noxious products. In the alternative, CD4+ T lymphocytes activated in sinus tissues might find their way to the lung and set up an inflammatory response.[20] Conceivably, cytokines released from diseased sinus tissues might activate circulating EOSs that would then find access to lung tissues. It appears quite possible that in the near future it may be possible to determine definitely whether sinus tissue or lung tissue first becomes inflamed and then to demonstrate the cellular mechanism responsible for the striking association between sinusitis and asthma.

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RELATIVE PROGNOSTIC IMPORTANCE OF HISTOLOGIC INVASION OF THE LARYNGEAL FRAMEWORK BY HYPOPHARYNGEAL CANCER

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This study assessed the relative prognostic importance of histologic invasion of the laryngeal framework by hypopharyngeal cancer. The laryngeal specimens and medical records of 55 patients found to have primary hypopharyngeal cancer between 1962 and 1988 were reviewed. Full 3-year follow-up information was obtained for 51 patients. The overall 3-year survival rate was 43% (22/51). The 3-year survival rate was 55% (17/31) without histologic invasion versus 25% (5/20) with invasion ($p < .05$). To assess how invasion affected survival rates in conjunction with clinical predictors, we divided the cohort into two groups (mild versus severe illness) based on the presence or absence of anemia and comorbidity. Only in the group with mild illness did histologic invasion provide additional prognostic information. These results demonstrate that the inclusion of clinical variables in predictions of prognosis can strikingly alter the prognostic importance of invasion of the laryngeal framework.

KEY WORDS — anemia, comorbidity, head and neck cancer, histology, hypopharyngeal neoplasms, prognosis, recurrence rate, squamous cell carcinoma, survival rate.

INTRODUCTION

For patients with cancer of the larynx and pharynx, the gross morphologic extent of the tumor is often cited as the single most important prognostic factor.[1] The American Joint Committee on Cancer[2] reports that neoplastic invasion of the ossified laryngeal framework is an anatomic variable that has independent prognostic value. Previous work by one of the authors supports this finding.[3] In that work, 42 total laryngectomy specimens from patients with glottic cancer and 56 total laryngectomy specimens from patients with transglottic cancer were examined. In the group of patients with glottic cancer, the 3-year recurrence rate for those patients whose specimens did not have invasion was 38%, whereas the 3-year recurrence rate was 72% for those patients whose specimens did have invasion. In the group of patients with transglottic cancer, the 3-year recurrence rate was also adversely associated with invasion (43% versus 57%).

The purpose of this study was to assess the prognostic importance of histologic invasion of the ossified thyroid or cricoid cartilage by cancer originating in the hypopharynx. In addition to tumor-based morphologic factors, prognosis for patients with cancer is also dependent on such clinical or patient-based factors as age[4,5]; gender[6]; nutritional status[5,7]; immune status[8,9]; symptom type, duration, and severity[10-16]; and the existence of other illnesses.[10,11,17] Therefore, to account for the relative prognostic impact of clinical factors, we collected information on both tumor and clinical factors in this study. Prognostic clinical factors were combined to create a two-category severity of illness index[18] to control for the impact of these clinical factors on outcome. With this type of analysis, the relative prognostic importance of invasion of the laryngeal framework was evaluated. In addition, the value of formally including clinical or patient-based variables in predictions of prognosis is discussed.

MATERIALS AND METHODS

Population Under Study. All patients treated by laryngectomy at Yale–New Haven Hospital (YNHH) between 1962 and 1988 for a cancer involving the hypopharynx were identified through the Yale collection of laryngectomy specimens. This collection was chosen as the source of our patient population because the anatomic extent of cancer in each laryngectomy specimen could be precisely determined from the serial sections made from each specimen.

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According to a technique described by Tucker and Smith,[19] each larynx had been serially sectioned in celloidin (until 1987) at 20-μm intervals. Beginning in 1987, the specimens had been sectioned in paraffin.

From the collection, 81 patients were identified to have cancer involving the hypopharynx. After review of the laryngectomy sections in conjunction with the medical records stored in the YNHH Medical Records Department, the Yale Tumor Registry, and the Yale Radiation Therapy Department, 26 of the 81 patients were excluded from further analysis. Of these 26 patients, 10 patients were excluded because they had failed irradiation as a first course of therapy, and 16 were excluded because the primary tumor had not originated in the hypopharynx.

The exclusion of these 26 patients left an inception cohort[20] of 55 patients with primary hypopharyngeal cancer treated with a total or partial laryngectomy as part of their first antineoplastic therapy at YNHH between 1962 and 1988.

Data Collection. For each patient in this study, information from the medical record was extracted onto a special form utilizing standard methods of collecting clinical data for predictions of prognosis for cancer and other chronic diseases.[20,21] A zero time was defined for each patient as the date of the first antineoplastic therapy directed against the cancer of the hypopharynx. The data extracted from the medical record for the pre–zero time interval included basic demographic data; symptom type, duration, and severity; other existing medical conditions; pertinent laboratory and radiographic studies; and an anatomic description of the primary tumor and nodal and distant metastatic spread. The criterion for anemia was a hematocrit value of 38 or less for men and women.

A course of antineoplastic therapy was defined as a sequence of one or more therapeutic modalities directed against a particular anatomic manifestation of cancer. The modalities available were surgical, radiologic, and chemical. Our coding form recorded all the components of each course of therapy, the pathologic reports of surgical margins and dissected lymph nodes, operative and postoperative complications, and the time between each course of therapy.

Symptom Classification. The type, duration, and severity of a patient's symptoms can indicate prognosis, because they reflect the clinical biology of a cancer. For example, a patient with systemic symptoms associated with a glottic cancer, such as fatigue or weight loss, would be expected to have a worse prognosis than a patient with glottal cancer whose symptoms had remained exclusively local, such as hoarseness. The duration of symptoms can indicate the rate of growth of a cancer, with a long duration of symptoms implying slow growth. Different symptoms can be classified according to their presumptive mechanisms and effects. This study adopted a classification scheme that has been used in a variety of studies of different cancers, in which symptoms were classified as local, systemic, or distant.[10,20]

Comorbidity. Comorbidity is any disease, illness, or condition other than the index disease under treatment or evaluation.[22] Prognostic comorbidity refers to any comorbidity so severe that it might be expected to impact on the patient's outcome independently of the index disease. In this study the term prognostic comorbidity was used for concomitant ailments that would be classified as "grade 3" comorbidity according to the Kaplan-Feinstein classification system.[23] The following are examples of ailments classified as grade 3 comorbidity: congestive heart failure or myocardial infarct within the past 6 months, recent stroke, marked pulmonary insufficiency (ie, cyanosis, carbon dioxide narcosis), uremia, hepatic failure (ascites, icterus), and severely decompensated alcoholism (more than one episode of delirium tremens or alcoholic seizures).

Anatomic Extensiveness. Tumor staging was updated to the criteria of the 1988 American Joint Committee[2] on the basis of the senior clinician's (J.A.K.) written description of tumor extent and degree of vocal cord fixation at the time of initial evaluation. If cord mobility was not assessed because of an inability to see the true vocal cords due to tumor mass or edema, or if cord mobility was described as being limited, the vocal cord was considered to be fixed.

Histopathology. Histologic invasion was characterized by invasion of the ossified thyroid or cricoid cartilages by hypopharyngeal cancer and was confirmed by the senior author. Sections from two laryngectomy specimens (Fig 1) demonstrate ossified laryngeal cartilage without and with invasion by hypopharyngeal cancer. A patient's pathologic node status was determined from pathologic reports of operative specimens. Pathologic node status was categorized as "positive" if tumor was described in one or more lymph nodes, and as "in soft tissue" if tumor was described in the soft tissues surrounding the lymph nodes.

Outcome. Each patient's medical record was inspected to obtain post–zero time information on recurrence, development of second primary tumors in the head and neck and other sites, and life survival.

Data Analysis. After all medical record and histopathology information was transferred to coding

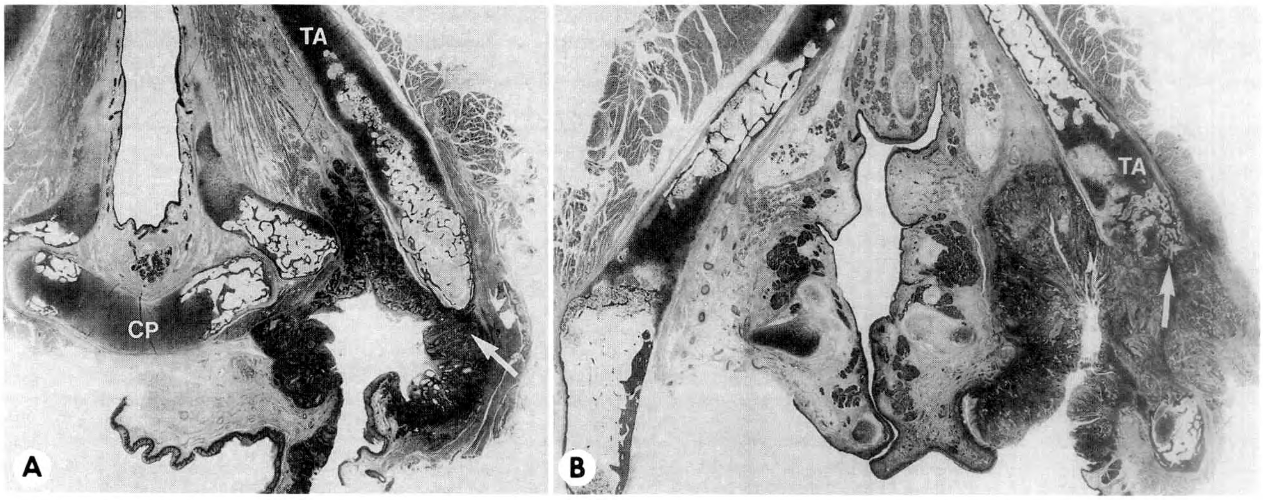

Fig 1. Sections from two laryngectomy specimens. A) Transverse section showing ossification of dorsal edge of thyroid ala (TA) and cricoid plate (CP), with no invasion by adjacent pyriform sinus carcinoma (arrow). B) Carcinoma (arrow) invading ossified dorsal edge of thyroid ala (TA).

forms, computerized data entry was performed by a professional data management company and subjected to double entry and verification. Sorting, tabulation, and statistical analysis were performed with the Statistical Analysis Software system (SAS Institute Inc, Cary, NC). Univariate analyses were used to evaluate baseline variables. Bivariate analyses, utilizing Fisher's exact tests, χ^2 analysis, and χ^2 for linear trend, were used to examine the prognostic impact of histologic invasion and other tumor and clinical variables. Two-tailed tests of significance were used. Because of the small sample size, a multivariable approach called conjunctive consolidation,[24] and not multiple logistic regression, was used to combine prognostic baseline clinical factors into a clinical severity index. The prognostic impact of invasion of the laryngeal framework was then assessed within categories of clinical illness. The clinical severity index was created, therefore, to control for baseline clinical factors that could confound the analysis of the prognostic importance of histologic invasion.

RESULTS

Baseline Classification and Survival Rates. The cohort of 55 patients consisted of 43 men and 12 women. The median age was 58; there was 45 whites, 6 blacks, 1 Hispanic, and 3 individuals of unknown race. All 55 patients had a nonrecurrent, biopsy-proven squamous cell carcinoma of the hypopharynx. Full 3-year follow-up was obtained for 51 patients. All 55 specimens had involvement of the pyriform sinus; 10 of these also had involvement of the posterior pharyngeal wall, and 6 had involvement of the posterior cricoid.

Table 1 shows the prognostic impact of selected baseline clinical variables on 3-year survival. Age, gender, symptom class, and symptom duration had little to no impact on survival. Prognostic comorbidity had the highest survival gradient (although not statistically significant); the 3-year survival rate for the 4 patients with prognostic comorbidity was 0%. Patients without anemia had a 3-year survival rate of 54%, versus 17% for those with anemia ($p < .05$). Ipsilateral vocal cord mobility was not significantly associated with survival. A stage II cancer was associated with a worse 3-year survival rate than a stage III cancer (40% versus 60%), and a stage IV cancer was associated with a worse 3-year survival rate than a stage III cancer (32% versus 60%); neither difference was statistically significant. Positive node status was associated with a worse 3-year survival rate when compared to a negative node status (50% versus 67%). When patients with nodal metastases that involved adjacent soft tissue were included with patients with nodal metastases that had remained intracapsular, pathologic node status gained statistical significance ($p < .01$). The 3-year survival rate of patients without histologic invasion of the laryngeal framework was 55%, versus 25% for those with invasion ($p < .05$).

Each larynx, except one, was removed by a total laryngectomy. As a first antineoplastic therapy, 11 patients were treated with surgery only, 24 with planned sequential preoperative irradiation followed by surgery, 17 with surgery and postoperative irradiation, and 3 with surgery, postoperative irradiation, and chemotherapy. Three-year survival rates by type of therapy were as follows: only surgery 44% (4/9, 2 patients lost to follow-up [LFU]); preoperative irradiation and surgery 39% (9/23, 1 patient LFU); postoperative irradiation and surgery 50% (8/16, 1

TABLE 1. THREE-YEAR SURVIVAL RATES ACCORDING TO EIGHT BASELINE AND TWO PATHOLOGIC VARIABLES OF CLASSIFICATION

Variable	*Patients*	*Survivors*	*LFU*	*3-y Survival Rate (%)*	*Survival Gradient**
Total population	55	22	4	43	
Age group					
≤51 y	15	6	0	40	
52-61 y	22	10	2	50	
>61 y	18	6	2	38	12% (NS)
Gender					
Men	43	17	4	44	
Women	12	5	0	42	2% (NS)
Symptom class					
Local	28	11	3	44	
Systemic	13	5	0	38	
Distant	14	6	1	46	8% (NS)
Symptom duration					
≤6 mo	41	19	0	46	
>6 mo	11	3	2	33	13% (NS)
Unknown	3	0	2	0	
Prognostic comorbidity					
Absent	47	22	2	49	
Present	4	0	0	0	49% (NS)
Unknown	4	0	2	0	
Anemia					
Absent	28	14	2	54	
Present	12	2	0	17	37% ($p < .05$)
Unknown	15	6	2	46	
Ipsilateral cord mobility					
Mobile	15	7	1	50	
Fixed	30	13	1	45	5% (NS)
Unknown	10	2	2	25	
TNM stage					
I	0	0	0	0	
II	6	2	1	40	
III	22	12	2	60	
IV	25	8	0	32	28% (NS)
Unknown	2	0	1	0	
Pathologic node status					
Negative	9	6	0	67	
Positive	24	10	4	50	
In soft tissue	9	0	0	0	67% ($p < .01$)
Not done or unknown	13	6	0	46	
Histologic invasion					
Absent	34	17	3	55	
Present	21	5	1	25	30% ($p < .05$)

χ^2 Used for linear trend where appropriate. Significance levels given by χ^2 test.

LFU — lost to follow-up, NS — not significant.

*Difference between highest and lowest survival rates in each category, excluding those patients unknown.

patient LFU); and surgery, postoperative irradiation, and chemotherapy 33% (1/3). These survival differences were not statistically significant ($p = .90$). Histologic invasion was found in 36% (4/11) of the patients treated only with surgery, 46% (11/24) of the patients treated with preoperative irradiation and surgery, 29% (5/17) of the patients treated with surgery and postoperative irradiation, and 33% (1/3) of the patients treated with surgery, postoperative irradiation, and chemotherapy. These differences were

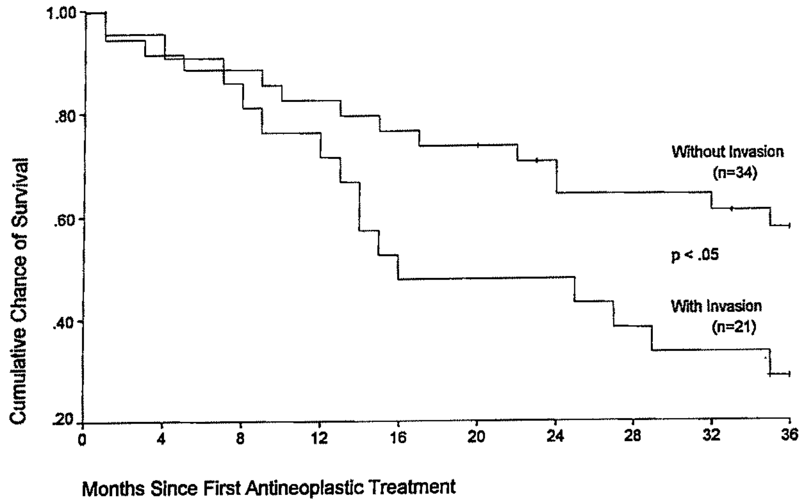

Fig 2. Observed survival for patients with and without histologic invasion. Tick marks represent patients who were alive at time of last follow-up.

not statistically significant ($p = .75$).

Figure 2 demonstrates the 3-year observed survival rate, utilizing the Kaplan-Meier life table analysis, for patients with and without histologic invasion. The 3-year cumulative chance of survival was 29% with invasion and 58% without invasion ($p = .037$, log-rank test; $N = 55$). The 3-year cumulative chance of recurrence was 63% with invasion and 52% without invasion ($p = .384$, log-rank test; $N = 55$).

Survival and Recurrence in Composite Clinical Illness Severity Stages. Because the clinical variables anemia and comorbidity were associated with survival, a clinical severity index was created to categorize, or stage, patients on the basis of these variables. This was done to assess the relative prognostic impact of histologic invasion. Because the number of patients in this study was too small for conventional multivariable analysis,[25,26] conjunctive consolidation techniques[24] were used to combine anemia, comorbidity, and histologic invasion for multivariable analysis. The clinical severity index was created by grouping patients with anemia and/or prognostic comorbidity into the clinical illness stage called "severe" and patients with neither anemia nor prognostic comorbidity into the clinical illness stage called "mild." Patients with an unknown hematocrit value and absent or unknown prognostic comorbidity were included in the mild illness stage. Presumably, if a patient had a low hematocrit value or severe comorbidity, this information would have been in the medical record.

TABLE 2. THREE-YEAR SURVIVAL RATES IN COMPOSITE CLINICAL ILLNESS SEVERITY STAGES

Stage	*3-y Survival*
Mild	20/37 (54%)*
Severe	2/14 (14%)
Total	22/51 (43%)†

Denominators — number of patients in each category, numerators — corresponding number of 3-year survivors.

*Four patients were excluded from analysis because 3-year survival status unknown.

†Survival gradient is 40%; $\chi^2 = 6.55$, $p < .025$.

Tables 2 and 3 present the 3-year survival and recurrence rates, respectively, for patients in the two different clinical illness stages. Individuals with mild illness, regardless of other factors, had a significantly improved 3-year survival rate compared to those individuals with severe illness (54% versus 14%, $p < .025$). The 3-year recurrence rate was 54% with mild illness and 80% with severe illness ($p = .27$).

Table 4 displays the relative prognostic importance of histologic invasion of the laryngeal framework within stages of clinical illness. For patients with mild illness the overall 3-year survival rate was 54%, but the 3-year survival rate for patients with histologic invasion was 29%, versus 70% for those patients without invasion ($p < .025$). In the group with severe illness the overall 3-year survival rate was so poor (14%) that histologic invasion was not significantly associated with a difference in survival (17% versus 13%).

Table 5 demonstrates the recurrence rates within

TABLE 3. THREE-YEAR RECURRENCE RATES IN COMPOSITE CLINICAL ILLNESS SEVERITY STAGES

Stage	*3-y Recurrence*
Mild	19/35 (54%)*
Severe	8/10 (80%)†
Total	27/45 (60%)§

All patients who died without recurrence within 36 months since their first antineoplastic therapy were excluded.

*Six patients excluded from analysis because tumor status unknown.

†Four patients excluded from analysis because tumor status unknown.

§Difference in recurrence rate is not significant ($p = .27$) by Fisher's exact test, two-tailed.

TABLE 4. THREE-YEAR SURVIVAL RATES IN CONJUNCTION OF CLINICAL ILLNESS AND HISTOLOGIC INVASION

	Clinical Illness Stage		
Histologic Invasion	*Mild*	*Severe*	*Total*
Absent	16/23* (70%)	1/8 (13%)	17/31 (55%)
Present	4/14† (29%)	1/6 (17%)	5/20 (25%)
Total	20/37§ (54%)	2/14 (14%)	22/51 (43%)

*Three patients lost to follow-up.

†One patient lost to follow-up.

§Difference in survival within mild class of illness between groups of histologic invasion (absent versus present) is statistically significant ($\chi^2 = 5.88$, $p < .025$).

stages of clinical illness. For patients with mild illness the 3-year recurrence rate was 43% without invasion and 75% with invasion (p = .08). For patients with severe illness the 3-year recurrence rate was 86% without invasion and 67% with invasion (p = 1.00, Fisher's exact test).

Conjunction of Histologic Invasion With Pathologic Node Status. Table 6 shows the prognostic impact of histologic invasion within each category of pathologic node status. In each category of pathologic node status with the exception of "in soft tissue," the 3-year survival rates were substantially lowered when histologic invasion was present. Sixty-nine percent (11/16) of the patients with histologic invasion and 85% (22/26) of the patients without histologic invasion had nodal metastases ($p = .22$, χ^2 test).

Clinical Predictors of Histologic Invasion. Table 7 shows the relationship between baseline vocal cord function and histologic invasion. The positive predictive value of a fixed ipsilateral and fixed contralateral vocal cord was, respectively, 47% (14/30) and 100% (7/7). That is, for patients with hypopharyngeal cancer there is a 47% chance of histologic invasion when the ipsilateral cord is fixed; there is a 100% chance when the contralateral cord is fixed. The negative predictive value of a mobile ipsilateral vocal cord and a mobile contralateral cord was, respectively, 87% (13/15) and 75% (15/20). This means that when the ipsilateral cord is mobile there is an 87% chance, and when the contralateral cord is mobile there is a 75% chance, that there is no histologic invasion.

TABLE 5. THREE-YEAR RECURRENCE RATES IN CONJUNCTION OF CLINICAL ILLNESS AND HISTOLOGIC INVASION

	Clinical Illness Stage		
Histologic Invasion	*Mild*	*Severe*	*Total*
Absent	10/23* (43%)	6/7† (86%)	16/30 (53%)
Present	9/12* (75%)	2/3* (67%)	11/15 (73%)
Total	19/35§ (54%)	8/10 (80%)	27/45 (60%)

All patients who died without recurrence within 36 months since their first antineoplastic therapy were excluded.

*Three patients excluded because tumor status unknown.

†One patient excluded because tumor status unknown.

§Difference in recurrence within mild class of illness between groups of histologic invasion (absent versus present) is not statistically significant ($\chi^2 = 3.16$, $p = .08$).

TABLE 6. THREE-YEAR SURVIVAL RATES IN CONJUNCTION OF HISTOLOGIC INVASION AND PATHOLOGIC NODE STATUS

	Histologic Invasion		
Pathologic Node Status	*Absent*	*Present*	*Total*
Negative	4/4 (100%)	2/5 (40%)	6/9 (67%)
Positive	9/16* (56%)	1/4† (25%)	10/20 (50%)
In soft tissue	0/3 (0%)	0/6 (0%)	0/9 (0%)
Not done or unknown	4/8 (50%)	2/5 (40%)	6/13 (46%)
Total	17/31 (55%)	5/20 (25%)	22/51 (43%)

*Three patients lost to follow-up.

†One patient lost to follow-up.

DISCUSSION

This study demonstrates that 3-year survival rates for individuals with cancer of the hypopharynx were significantly associated with histologic invasion of the laryngeal ossified framework. This finding is consistent with other studies of the prognostic impact of histologic invasion in patients with head and neck cancer. The exact reasons for this finding are unknown. It may be that histologic invasion is a sign of a more biologically aggressive cancer. Histologic invasion may also allow greater hematologic and lymphatic spread, with a resultant poorer prognosis for patients with such a finding — although, in this study, tumors that had histologic invasion did not have rates of nodal metastasis (a measure of biologic aggressiveness) significantly different from tumors that did not have histologic invasion.

TABLE 7. ASSOCIATION OF VOCAL CORD MOBILITY AND HISTOLOGIC INVASION

	Histologic Invasion		
Cord Mobility	*Absent*	*Present*	*Total*
Ipsilateral			
Fixed	16	14	30
Mobile	13	2	15
Unknown	7	3	10
Contralateral			
Fixed	0	7	7
Mobile	15	5	20
Unknown	19	9	28

Many articles have demonstrated that nonmorphologic, clinical factors can provide additional prognostic information for cancer patients. Anemia and comorbidity were the most important clinical predictors of survival in this study. Anemia may be a reflection of the aggressive nature of a cancer and/or of the general health status of the patient. Multiple mechanisms, such as occult blood loss or iron deficiency due to dysphagia or anorexia, could explain why a highly aggressive cancer would cause anemia before a less aggressive cancer.[27,28] Alternatively, other coexisting ailments or poor nutritional status as a result of excessive alcohol intake could explain anemia in some patients. These causes of anemia might decrease the patient's ability to kill tumor cells. Anemia may, therefore, reflect a general weakness to combat cancer due to an individual's general poor state of health.

A reflection of the general state of health of the patient is comorbidity. Although not a feature of cancer itself, comorbidity is an important attribute of the patient with cancer. The patient's concomitant disease(s) other than the cancer can strongly affect the patient's survival, tumor response, and treatment.

The information obtained from clinical variables and whole-organ examination of the larynx can affect postsurgical prognostic estimates and treatment recommendations. The prognosis for patients with mild illness and without histologic invasion is good. Since the prognosis is very poor for patients with hypopharyngeal cancer who have severe illness, regardless of invasion, palliative and not curative treatment might be more appropriate. Because vocal cord mobility provides insight into the histologic extent and aggressiveness of the tumor, modifications in surgical therapy might be made on the basis of the preoperative mobility of the affected cord. For example, if the ipsilateral cord is mobile and other clinical and radiographic studies indicate that there is no invasion of the laryngeal framework, a partial laryngopharyngectomy, instead of a total laryngopharyngectomy, might be considered. If the contralateral, or even the ipsilateral, cord is fixed, a total laryngopharyngectomy may be the more appropriate surgical procedure.

The number of patients included in this study is small, and complete baseline and follow-up information was not obtained on all patients. Because of these limitations, the results should be viewed as preliminary. Nevertheless, this study reports on the largest number of patients included in a whole-organ study of hypopharyngeal carcinoma and provides additional support for the concept that prognosis for patients with head and neck cancer is based on *both* tumor and patient factors. It is anticipated that future research, utilizing previously described methodology for the evaluation of putative prognostic factors,[29,30] will further confirm the value of formally including patient-based variables in predictions of prognosis.

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VASCULAR EFFECTS OF SUSTAINED-RELEASE FIBROBLAST GROWTH FACTORS

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Since the half-life of most angiogenic growth factors is several hours or less, sustained-release delivery would be optimal for their future clinical use. Two fibroblast growth factors, basic fibroblast growth factor (bFGF) and endothelial cell growth factor (ECGF), were delivered in two sustained-released modalities (poloxamer 407 and a gelatin sponge [Gelfoam]) to attempt to increase soft tissue vascularity. In vitro bioactivity of ECGF-poloxamer formulations was also tested on endothelial cell cultures. Among vascular-compromised skin flaps in rabbits, ECGF-poloxamer ($N = 26$), bFGF-poloxamer ($N = 5$), ECGF-poloxamer ($N = 9$, irradiated), and bFGF-Gelfoam flaps ($N = 22$) did not demonstrate significant differences in viability and vascularity compared to controls ($p > .05$). Irradiation had a detrimental effect on both flap vascularity and viability ($p = .02$). Future efforts for sustained delivery of angiogenic proteins are critical in order to make them clinically useful in wound healing.

KEY WORDS — growth factors, neovascularization, surgical flaps, wound healing.

INTRODUCTION

Over the last 20 years, angiogenic growth factors have been identified that have the ability to induce neovascularization. The fibroblast growth factor (FGF) family is especially promising, since they are some of the most potent angiogenic growth factors known. However, since the half-life of most angiogenic agents is several hours or less, sustained-release modalities for delivery would be optimal to prolong their exposure to wounds and maximize their effects.

The FGFs would especially be useful in wound healing conditions of delayed vascularization, such as in irradiated soft tissue. This finding has been supported by the discovery that FGFs accelerate cellular migration in irradiated endothelial cell cultures.[1] When endothelial cell cultures were subjected to increasing doses of radiation, basic FGF (bFGF) significantly contributed to maintaining cell proliferation and cell mobility.[1]

Past studies have explored the sustained effects of a crude form of FGF, endothelial cell growth factor (ECGF), which contains acidic FGF and bFGF, combined with a gelatin sponge (Gelfoam; UpJohn Co, Kalamazoo, Mich). When this combination was added to vascular-compromised soft tissue, vascularization appeared to be accelerated in both irradiated and nonirradiated tissue.[2] Unfortunately, the optimal kinetic release of the FGFs contained in Gelfoam could not be technically controlled. Also, the rate of FGF release from Gelfoam is not known in vitro. In another study using Gelfoam, acidic FGF induced a significant angiogenic response when placed in multiple tissue sites compared to Gelfoam controls.[3] In several animal studies, FGF injected into skin flaps has been shown to either increase viability or have no effect on tissue viability.[4-8]

At present, much research is needed to design effective vehicles for delivering peptide growth factors to soft tissue. The pluronics, such as poloxamer 407, are very promising vehicles. Poloxamer 407 is a water-soluble, nontoxic block copolymer, capable of releasing biologically active proteins in their physi-

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ologically active form at a constant (zero order) rate.[9-11] Poloxamer 407 has been demonstrated to release biologically active proteins (recombinant interleukin-2, urease, transforming growth factor–α) in a sustained-release fashion in animals and in vitro.[9,10,12,13] By investigating the efficacy of sustained-release FGF in soft tissue, a provocative means of pharmacologic intervention will be explored to increase vascularity in a desired area.

The present study was designed to do the following.

1. To determine the kinetics of release of bFGF from poloxamer 407 in vitro.
2. To investigate the in vitro bioactivity of a form of FGF with poloxamer 407.
3. To investigate if forms of FGF released from poloxamer 407 and Gelfoam can expedite soft tissue vascularization.
4. To determine, in the setting of delayed wound healing, if vascularization can be enhanced by a form of FGF released from poloxamer 407.

MATERIAL AND METHODS

Fibroblast Growth Factors. Recombinant bFGF is a basic polypeptide (molecular weight = 17,500) cloned from human pituitary gland cells capable of inducing endothelial cell replication at 3 ng/mL.[14] Since the amino acid sequences of the FGFs are extremely well conserved among different species, the activity of bFGF is not species-specific. At least 1.1×10^4 to 1.3×10^4 ng/cm^2 was formulated to be released in a sustained manner over a 3-day period under vascular-compromised skin flaps. This peptide was donated by Synergen, Inc (Boulder, Colo). Biologic activity was confirmed by Synergen on Swiss 3T3 cloned D-1 mouse fibroblasts obtained from Dr Elaine Raines, University of Washington, Seattle, Wash (ED50 = 0.5 to 4 ng/mL). (The ED50 is the effective dose at which there is 50% activity.) When applied with Gelfoam, bFGF (250 μg) was reconstituted in 4 mL of phosphate-buffered saline solution.

Endothelial cell growth factor (Organon Teknika Corp Biotechnology Research Institute, Rockville, Md) is a neural extract derived from bovine hypothalamus mitogenic to endothelial cells at concentrations of 150 μg/mL. It is a crude form of FGF consisting of acid FGF and bFGF with their peptide fragments. The biologic activity of ECGF was confirmed on human umbilical cord vein endothelial cell lines by Organon Teknika Corp before application (ED50 = 15 to 30 μg/mL). Twenty-eight units of porcine heparin sodium (Elkins-Sinn Inc, Cherry Hill, NJ) was added with ECGF before incorporation in poloxamer 407. Heparin was added to ECGF to make it more chemically stable and to increase its biologic potency.[15,16]

Poloxamer Vehicle. Poloxamer 407 is a water-soluble, nontoxic block copolymer consisting of poly-(oxyethylene) and poly(oxypropylene) units. The copolymer is capable of releasing biologically active proteins at a constant (zero order) rate.[10] Poloxamer 407 (20% to 35% wt/wt) has the unique property of existing in the liquid state at room temperature, but forming a semisolid gel at body temperature. The polymer acts as a viscosity-enhancing excipient that prolongs the diffusion of the protein from the matrix. The rate of release of the protein of interest can be modulated by altering the formulation's viscosity. This is accomplished by increasing the percent weight per weight (%wt/wt) of poloxamer 407 in the protein–poloxamer 407 formulation.

Technique for Protein Incorporation and Release. Fibroblast growth factor was incorporated into poloxamer 407 by methods previously reported for inclusion of other biologically active macromolecules.[9,10,12] Briefly, for the in vitro release studies employing bFGF, 155 μg of bFGF was dissolved in a sufficient volume (approximately 0.5 mL) of pH = 7.0 phosphate-buffered saline contained in a tared 8-mL glass vial. Poloxamer 407 was then added to yield a final polymer concentration of 20%, 30%, and 35% (wt/wt) in the bFGF–poloxamer 407 formulation. Three such formulations were prepared for each of the polymer concentrations evaluated. A set of three vials that contained only poloxamer 407 at concentrations of 20% wt/wt, 30% wt/wt, and 35% wt/wt were also prepared to serve as controls. The vials were then placed on ice overnight to facilitate dissolution of the poloxamer 407 according to methods previously reported.[9,10,12]

In Vitro Release of FGF. Release of FGF was assessed in vitro by means of a membraneless diffusion system as described previously.[10] In brief, 1 mL of pH = 7 phosphate-buffered saline was layered directly above and in direct contact with the gelled protein-poloxamer matrices. All vials were then rotated on an orbit shaker at 150 rpm and the receptor phase fraction was completely removed and replaced with 1 mL of fresh buffer hourly for 8 hours. The 1-mL receptor phase fractions from both the bFGF–poloxamer 407 formulations and the control poloxamer 407 matrices were first weighed in additional tared 8-mL glass vials and then allowed to evaporate to dryness. The weight of the poloxamer 407 released into each receptor phase fraction was calculated under the assumption that the weight of bFGF re-

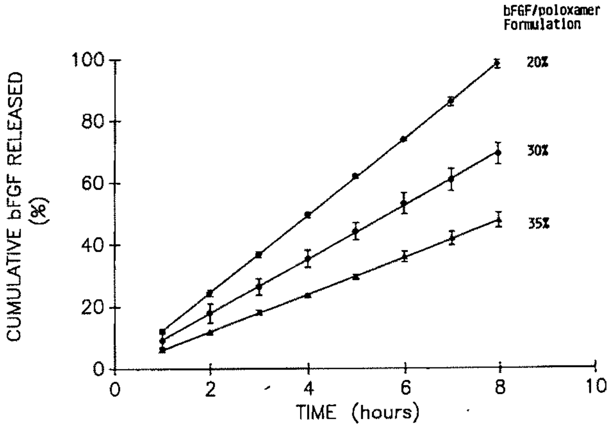

Fig 1. Cumulative percentage of basic fibroblast growth factor (bFGF) released over time in vitro from poloxamer 407 carriers (20%, 30%, and 35%).

TABLE 1. BASIC FIBROBLAST GROWTH FACTOR IN VITRO RELEASE STUDY

	% bFGF Released After 8 h		*Time for 100% Release (h)*
	Mean	*SD*	
Formulation 1 (20% wt/wt poloxamer)	97.8	1.4	8.2
Formulation 2 (30% wt/wt poloxamer)	68.9	3.5	11.6
Formulation 3 (35% wt/wt poloxamer)	47.6	2.4	17.9

Data describe cumulative percent of basic fibroblast growth factor (bFGF) released after 8 hours in vitro and extrapolated time required for 100% of bFGF to be released in vitro.

leased was negligible compared to the weight of poloxamer 407 released. As an example using the 20% wt/wt bFGF-poloxamer matrices, the total poloxamer amount in an individual matrix was 2 x 10^5 μg, compared to 155 μg of bFGF.

The above study was conducted in conjunction with a microprotein assay to quantitate the amount of poloxamer 407 and bFGF released versus time in vitro. Results of the above study demonstrated that the concentration of poloxamer 407 in an individual 1-mL receptor phase fraction was relatively constant throughout the 8-hour release period. Thus, a calibration curve was constructed for detection of bFGF that employed a medium containing various amounts of bFGF (0 to 20 μg) and the mean value of the concentration of poloxamer 407 determined in each receptor phase fraction for each concentration of poloxamer 407 evaluated. The microprotein assay kit (Pierce, Rockford, Ill) employed bicinchoninic acid (BCA) to detect the concentration of bFGF. The spectrophotometric assay was based on absorbance values obtained at 562 nm following the combination of bFGF and copper ion with BCA. Absorbances were compared to the calibration curve constructed with the bFGF and poloxamer 407 solution medium. The concentrations of bFGF in the receptor phase fractions were then determined. The data were expressed as the cumulative percentage of bFGF released from the matrices versus time, as depicted in Fig 1 and Table 1.

In Vitro Bioactivity Assay. The effect of poloxamer 407 on the growth of human endothelial cells in culture was assessed in vitro. Endothelial cell growth factor was dissolved in a solution of poloxamer 407 to yield final surfactant concentrations of 0%, 0.00005%, 0.0005%, 0.005%, 0.05%, 0.5%, and 5% wt/wt. The final concentration of ECGF in each well was 175 μg/mL. The assay was then performed as described in the following steps. Human endothelial cells were harvested, counted, resuspended to 5 x 10^4 cells per milliliter, and plated in 0.1-mL wells in a 96-well flat-bottom microtiter plate. To the wells, either 0.1 mL of the ECGF standard prepared in media (final concentrations of 50, 100, 150, and 200 μg/mL) or unknowns were added in quadruplicate. Negative controls contained media without ECGF. The plate was incubated for 5 days in a 37°C, 6% carbon dioxide (CO_2) humidified incubator. After incubation, the wells were aspirated, incubated for 4 hours with 0.1 mL per well of a 1-mg/mL MTT solution in Tris-buffered saline, pH 7.2, containing 10% glucose in a 37°C, 6% CO_2 humidified incubator. After the incubation wells were aspirated, the formazan precipitate was solubilized within 0.1-mL isopropanol wells; and their absorbance at 540 nm with a 690-nm reference was determined.

Experimental Design of Animal Model. Sixty-two New Zealand white rabbits (3 to 4 kg) were divided into four major groups. Group 1 (N = 26) had ECGF incorporated into poloxamer 407. Group 2 (N = 5) had bFGF within poloxamer 407. Group 3 (N = 9) had ECGF incorporated into poloxamer 407 and were irradiated. Group 4 (N = 22) had bFGF in Gelfoam. All animal use was approved by the Animal Care, Use and Research Studies Committee, Minneapolis Medical Research Foundation (NIH #A3875-01).

Animal Model. Full-thickness island skin flaps (4 x 2 cm) created on the rabbit ear dorsum were used as the soft tissue model, since their survival is dependent on adequate vascularization. To document the degree of peripheral neovascularization taking place, a Silastic sheet (5 x 3.013 cm; Dow Corning Corp, Midland, Mich) was placed underneath the flap to allow for only peripheral flap neovascularization to occur (Fig 2[17]). Two days after flap construction, the central vascular pedicle (artery and vein) was transected and cauterized to induce further vascular compromise. Similar to a previous published tissue model,

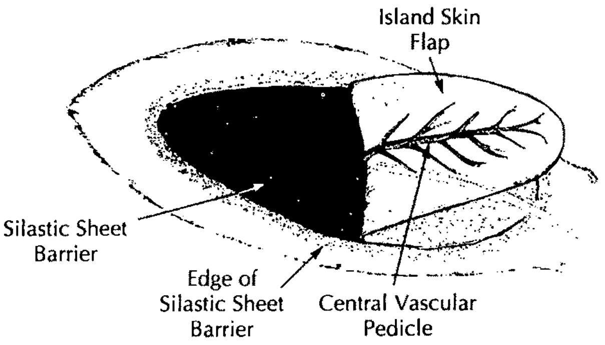

Fig 2. Rabbit skin flap model with Silastic (Dow Corning) barrier over flap bed to document peripheral vascularization. (Reprinted by permission.[17])

vascularization and skin flap survival was quantitatively documented by angiographic and surface viability methods as described in Methods, below.[2]

Irradiation. In group 3, 9 rabbits were anesthetized and secured in a retention device to allow the dorsal aspect of both auricles to be exposed on a flat surface. Enclosed in a field size of 20 × 24 cm, the auricles were irradiated, with the rest of the body of each rabbit shielded with a lead apron (4 mm thick). Both ears were irradiated simultaneously with an orthovoltage x-ray machine (Philips Medical Co, Shelton, Conn). The irradiation parameters were 250-kV peak, 15 mA, filtration of 1.0 mm aluminum plus 0.35 mm copper. The dose rate was 0.954 Gy/min and the skin-to-source distance was 60 cm. A single total dose of 15 Gy was given and confirmed with standard thermoluminescent dosimetry. Following irradiation, the rabbits were housed for 6 to 7 weeks before undergoing surgery. The radiation dose of 15 Gy was chosen on the basis of previous reports showing delayed vascularization in rabbit ear wound chambers.[18] A 6- to 7-week time delay before surgery was selected to avoid early soft tissue radiation effects, which occur 7 to 30 days postirradiation[19] (Fig 3).

Methods. For all groups 1 through 4, 62 island skin flaps were created on the rabbit ear dorsum with the sterile Silastic barrier secured between the flap and the perichondrial bed. To control for healing variability between animals, one ear of each rabbit served as the control, with the opposite side serving as the tested ear. In groups 1, 2, and 3, the bFGF-polymer (4 mL) or ECGF-polymer gel combination (4 mL) was applied on the underside of the flap, with the flap sutured back to its original position. In group 4, the bFGF in phosphate-buffered saline solution (4 mL) was added to a gelatin sponge (Gelfoam) underneath the flap. The flap was then sutured back to its original position. On the opposite ear, the repeat procedure was performed in the same fashion using phosphate-buffered saline (4 mL) integrated within the poloxamer (groups 1, 2, and 3) or Gelfoam (group 4). Two days after flap construction, the central vascular pedicle was transected and cauterized with the flap monitored for an additional 8 days. If any signs of seroma became evident underneath the flap, needle aspiration was performed and documented. Before each of the procedures, each rabbit was given intramuscular xylazine hydrochloride (9 mg/kg), atropine (0.16 mg), and ketamine hydrochloride (40 mg/kg), in addition to intramuscular chloramphenicol sodium (30 mg/kg).

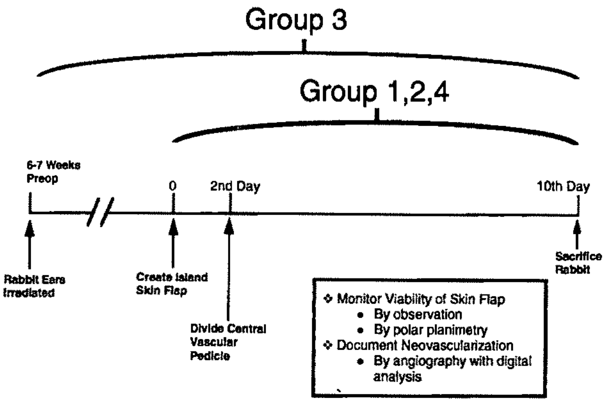

Fig 3. Time frame for each flap in groups 1, 2, 3, and 4 (see text).

Ten days after flap construction, the viable surface areas were traced with clear templates and quantified by polar planimetry (Zeiss Interactive Digital Analysis System). The percentage of tissue viability remaining on each flap was calculated by dividing the final viable area by the total flap area.

Flap angiograms were performed on all rabbits on the 10th day after flap construction. Under anesthesia, both common carotid arteries were cannulated and porcine heparin (1 mg/kg) was injected. Thirty percent micropaque in normal saline was continuously infused at 140 mm Hg, followed by 30% micropaque with 10% formalin via a manometric pump system described by Erol et al.[20] A femoral arteriotomy was performed to exsanguinate the animal. Thereafter, the ear was fixed in 10% formalin for a minimum of 2 days.

Digital angiographic analysis was performed on all flaps. For angiographic analysis, the flaps were peeled off the auricles and mammography techniques were used to magnify their images (1.5×) to maximize resolution of flap neovascularity. All angiograms were photographed with a dedicated stationary mammography system, a 5:1 reciprocating grid, and a 0.1-mm focal spot molybdenum target by means of Kodak OMI film with a Kodak Min-R screen, 20 mA/s, 23 kilovoltage peak for 0.20 second. In a blinded fashion, the angiograms were quantified by

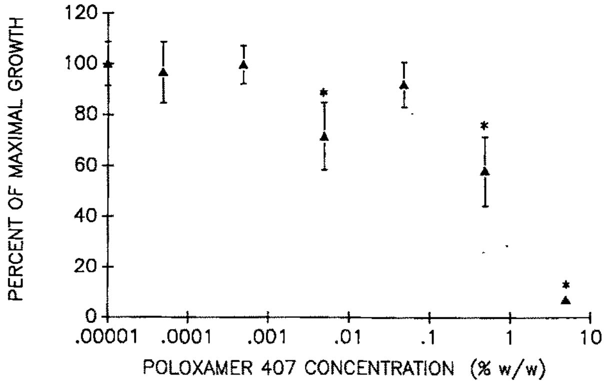

Fig 4. In vitro effects of poloxamer 407 concentration on biologic activity of endothelial cell growth factor for endothelial cell growth. Each measurement was performed in quadruplicate, with SD shown. Asterisks — significant difference ($p < .05$) compared to control solutions.

displaying each image on an International Image Processor (model 75) coupled to a Mass Comp MC 535 minicomputer. The flap vascularity was digitalized by the micropaque contrast color present in the perfused vessels. For statistical comparisons, vascularity and viability data were analyzed with a Kruskal-Wallis one-way analysis of variance.

RESULTS

In Vitro Release Study. The sustained release of bFGF from poloxamer 407 proceeded at a constant (zero order) rate at 22°C over the 8-hour test period (Fig 1 and Table 1). It is important to note that the observed bFGF released from poloxamer 407 in vitro does not necessarily reflect the bFGF amounts released in soft tissue. We estimate that the bFGF release in soft tissue probably occurs at a slower rate, because in vitro release studies have greater receiving fluid volumes to allow for greater diffusion.

Since ECGF contains bFGF and acidic FGF with its peptide fragments, the release of bFGF from the ECGF polymer would probably still show zero order kinetic release. The other proteins such as acidic FGF and their peptide fragments would also be expected to be released in a zero order fashion. However, the proteins of lower molecular weight, while still exhibiting a zero order release rate, would be expected to diffuse from the poloxamer matrices at a much faster rate.

Since normal soft tissue neovascularization begins at 3 days, an early sustained presence of FGF could allow the vascular process to occur earlier. Thus, the polymer was tailored to deliver at least 11.0 to 13.0 $\mu g/cm^2$ of bFGF over a 3-day period. The sustained 3-day release of the angiogenic agents was chosen to be most optimal because at this time interval, neovascularization is about to begin.[21] This concentration was selected from previous confirmed data in rodents, showing that a bFGF concentration greater than 1 $\mu g/cm^2$ clinically enhanced vascularization in diabetic mice.[22]

In Vitro Bioassay. Figure 4 illustrates the effects of increasing poloxamer 407 concentration on the biologic activity of ECGF. Each data point represents the mean value of four determinations. As the concentration poloxamer 407 was increased, the capacity of ECGF to stimulate growth of endothelial cells decreased. For example, at a concentration of 5% wt/wt poloxamer 407, 93% inhibition of growth was observed. Because results from in vitro cell culture assays do not necessarily predict expected outcomes in vivo, we proceeded with our studies designed to evaluate neovascularization and wound healing in vivo. Moreover, only nanogram quantities of FGF are required to effect endothelial cell growth in vivo.[23]

Animal Model. In the flap animal model, all flaps became cyanotic after vascular pedicle ligation on the second day. The viable and nonviable portions of the skin flap became evident over 7 days. As reported in previous studies using this model, a high seroma rate was present.[2] The seroma rate ranged from 65% (group 1) to 100% (group 2) in the treated flaps and from 50% (group 4) to 78% (group 3) in the control flaps. The presence of seromas did not appear to correlate with decreased skin viability clinically. Hematomas were not evident by needle aspiration.

Table 2 summarizes the viability and vascularity results in each group. Trends of increased viability

TABLE 2. FLAP VIABILITY AND VASCULARIZATION

			Mean Viability (%)		*Mean Vascularization (Pixel Units)*	
Group	*Growth Factor*	*Carrier*	*Treated*	*Control*	*Treated*	*Control*
1	ECGF (N = 26)	Poloxamer	12.1 ± 4.2	9.2 ± 2.0	5.0 ± 1.4	4.5 ± 1.3
2	bFGF (N = 5)	Poloxamer	5.4 ± 1.0	5.9 ± 1.6	10.4 ± 4.0	12.5 ± 8.6
3	ECGF (N = 9, irradiated)	Poloxamer	1.3 ± 1.0	1.0 ± 0.7	1.2 ± 0.5	1.8 ± 1.5
4	bFGF (N = 22)	Gelfoam	12.9 ± 1.7	10.6 ± 1.9	13.7 ± 3.3	7.8 ± 1.9

Data are mean ± SEM.

ECGF — endothelial cell growth factor, bFGF — basic fibroblast growth factor.

with the ECGF flaps (group 1) and increased vascularization and viability with the bFGF-Gelfoam flaps (group 4) were apparent. However, these increases were not statistically significant ($p > .05$). No statistically significant differences in viability and vascularity existed between treated and control flaps in these groups. Among the irradiated ECGF-poloxamer flaps in group 3, no statistically significant differences were evident in viability and vascularity between the treated and control flaps ($p > .05$). Since neither the viability nor vascularity data were distributed in a normal Gaussian distribution, nonparametric statistical analysis was performed by a Kruskal-Wallis one-way analysis of variance.

To determine if various amounts of ECGF incorporated into poloxamer 407 had any effect on flap viability and vascularity, 20% (wt/wt) poloxamer 407 matrices had been loaded with 28.8 mg, 57.6 mg, and 150 mg of ECGF. As determined by Kruskal-Wallis one-way analysis, a dose effect with regard to both vascularization and viability was not observed ($p > .05$) by increasing the amount of ECGF within the poloxamer matrix.

Overall, irradiation had a detrimental effect on both flap vascularity and viability. For the irradiated versus nonirradiated controls, irradiation significantly decreased both vascularity ($p = .02$) and viability ($p < .01$, Kruskal-Wallis one-way analysis of variance). In addition, among the ECGF-poloxamer–treated flaps with the same concentration (15 mg), irradiation significantly decreased viability ($p = .008$) and vascularity ($p = .05$; Mann-Whitney U test).

DISCUSSION

In this study, the FGFs bFGF and ECGF were delivered in two sustained-released modalities (poloxamer 407 and Gelfoam) to attempt to increase vascularity in a desired soft tissue area. Poloxamer 407 was tailored to give a constant (zero order) rate of sustained release of bFGF by altering the viscosity of the bFGF-poloxamer formulation. In addition, the in vitro bioactivity of ECGF-poloxamer formulations was tested on endothelial cell cultures. Unfortunately, as the poloxamer 407 concentration increased, the capacity of ECGF to stimulate endothelial cell growth decreased. In the vascular-compromised island skin flap model, ECGF and bFGF within poloxamer 407 did not demonstrate a statistically significant difference in viability and vascularity compared to controls. In the irradiated model, ECGF incorporated in poloxamer 407 also did not give a significant difference in viability and vascularity compared to controls. A trend existed showing increased vascularity of bFGF with Gelfoam, but this was not statistically significant.

Several reasons are possible for the inability of bFGF and ECGF to increase vascularity in this model.

First, the poloxamer and Gelfoam carriers inhibited the vascularization process. The in vitro endothelial cell bioassay that tested poloxamer formulations at various concentrations demonstrated inhibition of endothelial cell growth. This phenomenon occurred most dramatically when the poloxamer concentration exceeded 5% wt/wt. It is possible that poloxamer 407 binds to the membrane of endothelial cells. On the other hand, in vitro bioassays do not necessarily correlate with in vivo results. Previous studies of poloxamer have shown biocompatibility of this polymer in rats, rabbits, and mice.[9,11,12] Gelfoam has been previously shown to be an effective FGF carrier to induce vascularization in rats and rabbits.[2,3] Additional studies are needed to fully investigate the in vivo effects of these carriers.

Second, the polypeptides were not released in biologically active forms. Proteins are very susceptible to changes in pH, ionic strength, temperature, shear-related stresses, etc, and thus are prone to loss in their biologic activity. However, previous studies by one of the authors (T.P.J.) using the model proteins urease and recombinant interleukin-2 have demonstrated that poloxamer 407 did not cause irreversible inactivation of either protein in vitro or in vivo.[9,10,12] In addition, it was reported by these same authors that poloxamer 407 protected both model proteins from thermal- and solution agitation–induced loss in biologic activity and did not result in changes in the secondary structure of either protein.[12,24,25] Recently, another study has demonstrated the ability of poloxamer 407 to successfully release TGF-α in a sustained-release, biologically active form.[13] Thus, it is unlikely that poloxamer 407 caused a change in the tertiary structure of either ECGF or bFGF with subsequent loss in mitogenic activity. The Gelfoam carrier has also been previously shown to be a promising vehicle for FGF release.[3,7,17]

Third, sufficient amounts of bFGF and ECGF were not present long enough or in high enough concentrations to effect vascularization. Previous studies have shown that bFGF is dose-dependent, and quantities of 1 μg/mL in collagen were sufficient to induce vascularization in animal models.[7,8] At 300 μg/mL, ECGF caused increased neovascularization in animals when contained in Gelfoam.[26] The FGFs appear to require only partial receptor occupancy to initiate DNA synthesis in vitro.[15]

With this animal model, seroma formation is unfortunately very prevalent. The seroma may have further diluted the FGFs' effective concentrations. The Silastic sheeting in this model is necessary in order to allow for only peripheral vascularization to take place. This sheeting makes it possible to quantitatively document the peripheral neovascularization occurring. An additional effect is that this barrier probably also inhibits lymphatic efferent outflow. It is interesting to note that in all of the bFGF- and ECGF-treated groups, a higher incidence of seromas was found compared to that in controls. There is a possibility that efferent flow was further delayed by the effects of these growth factors. At present, it is not known how FGFs influence lymphatic vessels in vivo.

Fourth, the release kinetics of the proteins from the polymer matrices tested were not in synchrony with the time at which these factors are normally released. The in vitro release kinetics associated with bFGF cannot necessarily be extrapolated accurately to the in vivo delivery of the growth factor. The sustained release of proteins in vivo was probably slower because the receiving fluid is much more limited for diffusion. At present, the optimal time sequence for release of angiogenic agents to maximize vascularization is not known. It would appear that an optimal time for an angiogenic stimulus to occur would be within 3 days after wounding, since neovascularization begins at 3 days.

Fifth, the flap vascular compromise occurred too early and prevented FGF and ECGF from mediating their intended physiologic response. In this study, the reason the flap vascular compromise was selected on day 2 was that we wanted to ensure that a significant ischemic insult was inflicted to the contralateral control flap in order to magnify any vascular or viability differences from the treated flaps. We also wished to maintain all confounding variables the same between the groups by compromising the flaps on day 2, so that the growth factor or its carrier was being evaluated. Since FGFs are competence factors, they are required to initiate the cell cycle from G-0 to G-1. Earlier studies have demonstrated that FGF administered as a single dose caused its intended effects in vivo.[27]

Much as in previous studies, preoperative irradiation appeared to decrease vascularity in the skin flaps.[19] In this study, skin flap viability also decreased with preoperative irradiation. Past findings have been mixed as to whether preoperative irradiation adversely affects flap survival.[19,28-30]

Many complex variables continue to make the therapeutic delivery of growth factors challenging. Pharmaceutical issues to be addressed include the stability of growth factors in formulation, and delivery of these proteins in an enzymatically hostile environment. Despite our present findings, exploring sustained-release modalities for angiogenic proteins in the future can have an enormous impact on wound healing. Examples of such controlled-release systems for large molecules include porous polymer matrices, collagen sponges, and degradable polymeric delivery systems.[31] Depending on the type of polymer, several parameters determine the rate of controlled release of a protein: the molecular size of the protein, the initial amount of protein incorporated into the sustained-release formulation, the protein solubility of the polymer matrix selected, the composition and molecular weight of the polymeric vehicle selected, and the dimensions and shape of the matrix.

The delivery of growth factors by sustained-release systems to enhance angiogenesis in soft tissue is actively being investigated throughout the world. Promising methods for growth factor delivery include encapsulation in liposomes, incorporation in extracellular matrix carriers, and integration in polymers.[32-35] Ideally, the protein of interest should remain biologically active when released, and its carrier should remain fairly inert in the host so that background inflammatory and immunologic changes are minimal.[36] Future efforts for sustained delivery of angiogenic proteins are critical in order to make the proteins clinically useful in wound healing.

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LEARNING DISABILITIES AND CENTRAL AUDITORY DYSFUNCTION

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Hearing loss, whether peripheral or central, compounds the communication and educational problems of the learning disabled student. A central auditory processing disorder uniquely interferes with both the input and integration of verbal information, further resulting in a potentially permanent cognitive dysfunction during the developmental period of acquisition of language. Illustrative cases are presented that indicate the panorama of cognitive dysfunction associated with the learning disabled status. Methods of evaluation and identification and diagnostic criteria are correlated with auditory, visual, and academic performance. Comments regarding clinical awareness, prompt recognition, and ensuing individualized remediation are submitted.

KEY WORDS — central auditory dysfunction, education, hearing loss, language, learning disability.

INTRODUCTION

This investigation considers the auditory function of the student with learning disability (LD) and the impact of specific auditory impairments on the functional, educational, and social performance of the involved individual. A number of children who are ultimately categorized as having LD first come to the attention of the otolaryngologist with suspicion of hearing loss due to inappropriate or inconsistent responsiveness to spoken communication, especially in the school environment.[1] After the standard otologic examination and comprehensive audiometry, these children are then evaluated for a central auditory processing disorder.

This paper will present four previously diagnosed cases of LD in individuals with unique auditory deficits to illustrate the variability of this syndrome. While numerous central auditory tests exist, we have chosen a battery designed to evaluate auditory performance as it is reflected in normal social communication. Other tests that employ synthetic or nonsense syllables were not included, because they are not representative of real speech.

Review of the literature indicates that the causes of LD are numerous and occasionally poorly defined. From a clinical standpoint, auditory perceptual disorders may coexist with other areas of cognitive and receptive dysfunction.[2] It is noted that familial patterns are observed in approximately 40% of LD cases.[3] Other findings include narcolepsy, essential hypersomnia, and attention deficit hyperactivity disorder. Maternal diseases within the family appear more frequently than by random chance, such as systemic lupus erythematosus, and the toxic effect of illicit drugs, smoking, and alcohol may, in addition, be causally related to the neurologic damage manifested as LD. Other perinatal and postnatal factors, including low birth weight, abnormal uterine contractility, fetal hypoxia, and hyperbilirubinemia, may indirectly contribute to LD. A spectrum of neurologic disorders, therefore, may be evident in conjunction with the LD. For example, 20% to 25% of persons with LD present an attention deficit disorder (ADD), while others exhibit less common problems such as Tourette's syndrome, seizure disorders, sensory integration dissociations, and obsessive-compulsive behavior.[4-6] In addition, social, emotional, and family problems are apparent in many of these individuals.[7]

A dysfunction in visual, auditory, or other sensory input or integration can potentially result in the disruption of the normal information processing sequence. Learning disability is described in the *Diagnostic and Statistical Manual of Mental Disorders*[8] (revised edition) of the American Psychiatric Association and includes disorders of academic skills, language and speech development and function, and motor skills.

The definition of LD is established by federal law. "Education for All Handicapped Children," Public Law 94192, includes the following: "Specific learning disabilities mean a disorder in the psychological processes involved in understanding or using language, spoken or written, which may manifest in an imperfect ability to listen, think, speak, read, write, spell or do mathematical calculation. Exclusion from this group is based upon organic deficits including visual, hearing, motor handicap, retardation, emotion disturbance, or environmental, cultural, or economic disadvantage."[4(p182)] Learning disability therefore encompasses those children who do not achieve aca-

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demically or on standardized tests in general, and those with more specific difficulties in reading, writing, and verbal responsiveness.

THEORIES AND DEFINITIONS

The schematic representation of language function proposed by Heilman et al[9] in 1976 depicts a progression from the initial auditory analysis to phonemic areas that then coordinate with the semantic field, and finally interface and interact with motor, speech, and visual association areas. Accordingly, discrete areas of involvement may produce identifiable and relatively specific functional impairments, including Wernicke's aphasia, sensory and anomic aphasias, Broca's aphasia, and reading difficulties with or without accurate comprehension of spoken and written language.[9-12]

One theory of language and auditory processing is based on reception and interpretation of the input signal. Deficits include poor attention to auditory information, inadequate suppression of unwanted stimuli, inability to attend in a noisy environment, decreased utilization of linguistic information, and limited short-term memory. A remedial approach to these areas of dysfunction was proposed by Laskey,[13] who summarized the goals of therapy as enabling the child to more effectively select, comprehend, respond to, and remember auditory information.

Our clinical experience suggests that there are numerous functionally discrete areas of injury or dysfunction in children with LD that negatively affect different levels of language function and produce varied and qualitatively different communication disorders. The authors emphasize that the descriptive label of LD is not a homogeneous entity and should not be used to classify all children with various degrees of academic failure or language involvement. There are numerous types of dysfunction involving the auditory processing system; these include a segment who fail the normal learning strategies because of divided and focused attention, automaticity, and disordered information processing.[14-18] Others may exhibit specific deficits such as those in audition, including the inability to understand speech in noisy environments or distorted or time-altered speech.

Utilization of redundancy factors in the auditory message, such as intonation, stress, rhythm, and phrasing, may be similarly faulty, further limiting verbal auditory skills. Dichotic test findings suggest that the impairment is a result of right hemisphere or interhemispheric auditory processing deficits that affect paralinguistic and pragmatic features of conversation.[19-21] A cortical disassociation theory was suggested by Welsh et al[22] based on the dichotic central auditory test results of dyslexic children. This study identified a failure of both dominant and weak ear integration and difficulty with suppression of unwanted ambient acoustic stimuli. Silver[4] described a disability in subtle sound difference discrimination that interferes with accurate perception of individual words and leads to confusion in conversation, difficulty with auditory figure ground (listening in noise), and auditory "lags" or delays in speech processing. The overall consequence of these dysfunctions is manifested as inattention, improper response, need for repetition of questions, and an apparent hearing deficit.

PATHOPHYSIOLOGY OF LEARNING DISORDERS

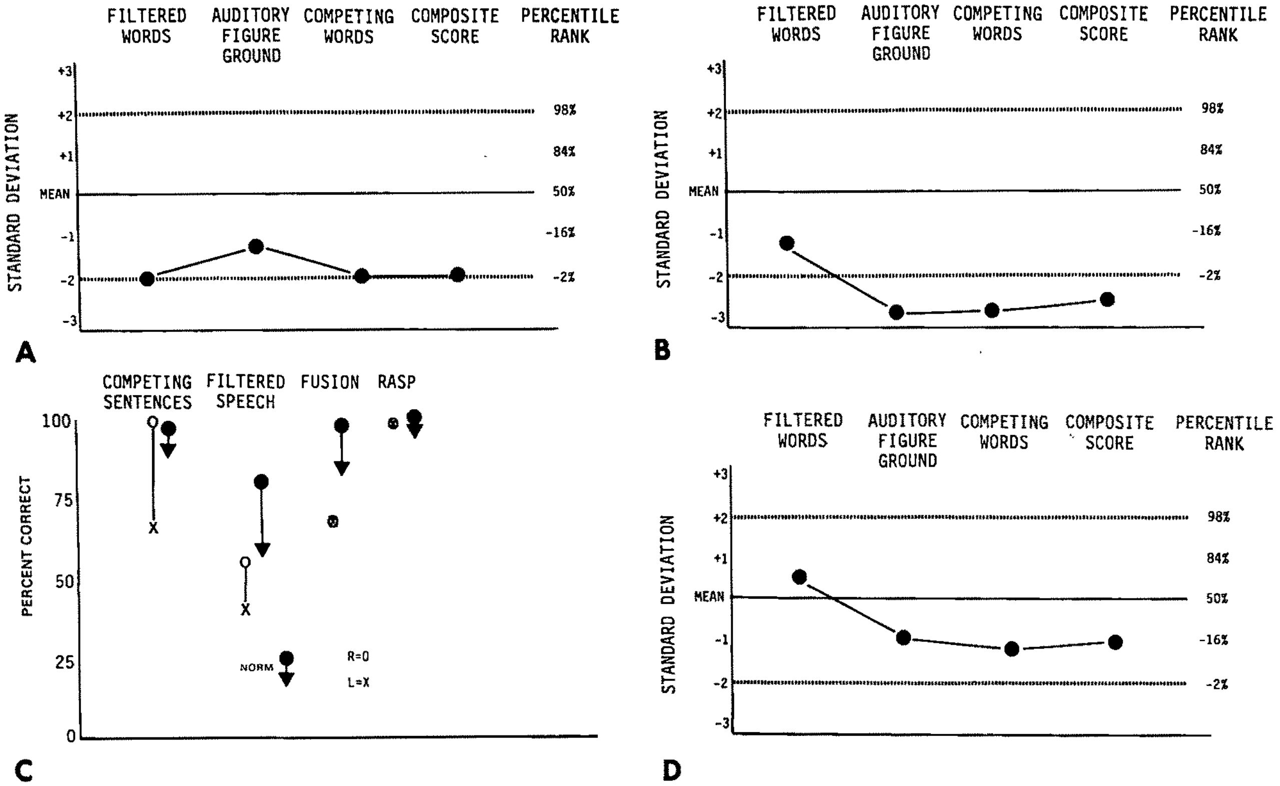
There is an extensive literature that suggests an asymmetry of the planum temporale,[23-25] an anomaly within the psychoarchitecture of the auditory association component in Wernicke's area.[26,27] Specific microscopic findings include ectopias, dysplasia, microgyria, and brain warts.[24] Other data suggest asymmetry in the language-related auditory association area that, in turn, is assumed to be causally related to the dyslexic syndrome. However, because of the differences in clinical manifestation, it is probable that the underlying pathologic mechanism is neither specific nor consistent within the LD group.

Electrophysiological measures have become a popular and effective tool in assessing many aspects of audition. These studies do not appear to identify a significant difference from normal values in the early auditory brain stem response[22] or in the middle latency response of children with LD or auditory processing disorders (unpublished data, Welsh et al). Data from Jirsa and Clontz[28] identify a significant latency increase for N1, P2, and P3 components of the late evoked potential as well as differences in interpeak latency intervals of P2 and P3. Further consideration of the P3 wave, which occurs approximately 300 to 700 milliseconds after stimulation, and its relationship to the psychologic state of the individual, suggests that this phenomenon may be the most sensitive to behavioral and cognitive problems.

In spite of these temporal and morphologic observations, the precise meaning of electroacoustic data is yet unknown; further, the tests are not sufficiently sensitive to localize areas of dysfunction or to indicate specific disease.

CLINICAL DATA

Clinical examples of four cases of LD are presented. The data consider auditory measurements, visual function, psychometric findings, and academic



A) (Case 1) Raw performance data are converted to standard deviation that identifies dysfunction in each test > –1 SD. Composite score of –2 SD from normal values summarizes overall functional level. B) (Case 2) Composite score of greater than –2 SD illustrates auditory dysfunction of central activities of bilateral identification (competing speech) and suppression (auditory figure ground). C) (Case 3) Norms are indicated by closed symbols, and individual performance by open symbols. Significant depression of performance is observed by filtered speech and binaural fusion test. RASP — Rapidly Alternating Speech Perception. D) (Case 3) SCAN test identifies impairment of function by > –1 SD in two of three tests, with composite rank of 16th percentile and composite score –1 SD.

achievement. Individual patterns of performance are examined, and the issue of maturation is explored where longitudinal data are available. After the standard examination and auditory assessment, central auditory testing was conducted. Standardized commercially available cassette tapes were presented via earphones in accordance with individual instructions for administration. The tests of Willeford[21,22] including competing sentences, low-pass-filtered speech and binaural fusion, Keith's SCAN (Screening Test for Auditory Perceptual Disorders),[29] and Beasley's 30% compression monosyllables were selected for this central test battery.

We have selected four unique LD individuals with essentially normal hearing bilaterally for the study. Cooperation, attention, and sufficient capacity to undergo testing were also essential prerequisites for inclusion in the investigation.

Case 1. A girl, 7 years 10 months of age, was assessed for hearing because of a reported sensory integration dysfunction noted in school, intolerance to loud noise, and a history of recurrent otitis media in infancy. She was the second of three children; the other two functioned within the normal range.

Physical examination disclosed a predominantly sinistral child without consistent handedness. Poor performance in fine and gross motor skills was noted. The basic hearing tests were within normal limits; the central testing that was performed at 7 years 10 months and again at 8 years 7 months revealed a significant dysfunction in auditory processing skills. On these occasions, the scores converted to a SCAN auditory age of less than 4 years 2 months, approximately 3 years 5 months and 4 years 5 months below expectations, respectively (see Figure, A). The visual examination identified poor tracking (following a moving target) and ocular convergence. Visual perceptual measurements indicated profound disability in the following areas: discrimination 0.4%, memory 0%, spatial relations 0.1%, form constancy 0%, sequential memory 0%, figure ground 0.1%, and closure 0%. The composite visual scores rank below the first percentile, and the perceptual quotient falls below the chart baseline for her age. The educational record revealed an inability to maintain regular class performance; consequently, she was assigned to a special ungraded program. Visual, auditory, and academic performance are all substantially below expected levels in this individual and are indicative of a multifocal dysfunction.

Case 2. A boy, 10 years 1 month of age, was examined for an apparent hearing loss based on the teachers' observations that verbal and behavioral responses were delayed or inappropriate to instruction and interrogation. The prenatal and postnatal courses were unremarkable; he is the third of five children. The parents and four siblings were reported to be academic achievers. During the interview he appeared slow in conversation, communicated with a limited vocabulary, and employed few words in response to questioning. Testing revealed satisfactory gross motor function but poorly performed fine eye and hand coordinated movements. The audiogram demonstrated a minor high-frequency hearing loss above 2 kHz with speech discrimination scores greater than 90% bilaterally. Degraded central auditory performance was observed on two separate occasions (see Figure, B). The second evaluation, at age 11 years 4 months, showed no improvement in competing words (first percentile), and the SCAN composite yielded an auditory age of 5 years 2 months. Improvement was noted on filtered words, with the current function at the 50th percentile. The scores on visual tests were uniformly low and indicated a functional level at 6 years 11 months, with a developmental lag of 2 years 2 months at the age of testing. The case illustrated an auditory and visual perceptual deficiency in conjunction with academic failure.

Case 3. A 10-year-old boy was referred as an academic underachiever. He was reported to exhibit a short attention span, poor comprehension, short-term memory deficit, and questionable hearing acuity. The family history is significant for an older brother who exhibits speech and motor problems and is classified as having LD. A recent ophthalmic evaluation revealed problems in lateral awareness, directional concepts, and auditory visual integration in the presence of normal vision. The auditory evaluation showed hearing and speech discrimination to be within normal limits. The Willeford battery was employed at this time and repeated at $13^1/2$ years. The patient scored significantly below normal on the filtered speech and binaural fusion subtests. The scores were within normal limits in the areas of competing sentences and rapidly alternating speech (see Figure, C). The SCAN test was administered (see Figure, D), and the auditory age equivalent for this $13^1/2$-year-old was approximately 7 years 5 months. The Iowa Tests of Basic Skills and Cognitive Abilities revealed a 64th percentile on verbal ability, 77th on quantitative, and 94th on nonverbal, suggesting an above-average child. With special educational support, including intensive tutoring, he was able to maintain class levels, although his performance was marginal to barely passable. This case demonstrates selective auditory and visual perceptual dysfunction associated with limited acadmic progress.

Case 4. A 23-year-old woman was initially surveyed audiologically at age $4^1/2$ years for poor speech, including articulation defects and limited vocabulary and verbal understanding. At that time and subsequently 4 years later, at age $8^1/2$, pure tone audiometry and speech discrimination values were normal. The family history failed to identify siblings with LD. The data from the Willeford battery at age $8^1/2$ fell within the normal range. While her auditory scores depicting auditory function appeared satisfactory, she commented on numerous occasions that she was often unable to understand instructions and required repetition before comprehension. Her academic career was supported by a reading tutor through the ninth grade. Currently she continues to read aloud while studying, and eventually, with greater personal motivation, she received a college degree. She commented at age 23 years about the distress associated with the slow integration of reading material and felt there was little improvement over the years.

The SCAN test fell within normal limits. Specific data at age 6 years 8 months, taken from the Illinois Test of Psycholinguistic Abilities (ITPA), revealed auditory perception of 5 years 8 months, visual reception 7 years 1 month, visual association 5 years, auditory association 6 years, auditory memory 4 years 10 months, and visual memory 6 years 6 months. The composite psycholinguistic age was 6 years.

In this brief overview of the patient's academic career and communication performance, it is apparent that the major deficit is in reading skills dating from early life. At this time and in the past, her hearing was measurably normal, yet her teachers insisted she could not hear, and a concern for amplification was entertained. These observations suggest an apparent audiolinguistic disorder, and while the linguistic skills appear to have progressed with maturation, there is continuing evidence of an unimproved reading problem and vague but realistic auditory complaints related to verbal reception.

DISCUSSION

The primary concern of this investigation is the role of hearing and central auditory function in specific LDs. In prior studies of a relatively homogeneous population with LD, we found fairly consistent results on all subtests measuring central auditory performance.[21,22] With this group, however, central auditory impairment ranged from relatively minimal to major communication disruption. It is characterized by, but not restricted to, delayed responsiveness, inadequate verbal interchange, a narrow vocabulary, or a failure to comprehend speech in a noisy, competitive, or distracting environment. Since the individuals in this study have normal hearing thresholds, a central auditory processing disorder is implicated. Three of the four patients demonstrate an auditory disability, extending from gross failure of function to relatively normal test performance. Analysis of the SCAN data reveals that these three patients display functional levels 2 SD below the norm. These findings correspond with the educational reports of hearing dysfunction and further support the clinical correlation described by Keith.[29] On the basis of the evidence presented in these illustrations, a child who had an apparent hearing loss not substantiated by peripheral audiologic tests should be investigated for central auditory processing disorder, since subsequent educational remediation is contingent on identification of hearing disability.[30] The sequel thereto is an individualized academic program for the specific needs of the student.

We have presented the scores of four individual cases rather than group LD statistics. For comparison, mean data deviation from norms and group performance of identified LD were presented in previous reports.[21,22] Group assessment data are indeed valuable, but because of the wide variations in performance, the information does not identify the functional level of the disabled individuals. A composite of the auditory, visual, educational, and psychologic profile is essential to the final assessment of the communication disorder and "the whole individual."

The most severe dysfunction is seen in patient 1, who is characterized by the lowest measurable levels. These data corroborate the observed and reported disabilities evident in school performance. Conversely, patient 4, a 23-year-old woman, suggests normal central hearing, although the recorded academic history firmly suggests diminished audition and/or moderate perceptual deafness. We assume that the central auditory tests utilized in these evaluations were insufficiently sensitive to identify the underlying communication dysfunction. Somewhere in the middle of this sensory deficit spectrum are the other two patients, with moderate impairment in communication.

The psychometric studies provide clues to the diagnosis and mechanism of auditory dysfunction as it interrelates with learning. Specifically, three of the students displayed auditory memory 2 years or more below chronologic age. While auditory scores alone cannot define LD, this factor is a significant component within the criteria for establishing the diagnosis of LD specified by the American Psychiatric Association.[8] Last, the data should be evaluated in conjunction with the paradigm of Ostrander[1] for the final diagnostic classification.

Perceptual and academic remediation poses a difficult problem. The type, locus, and effect of the dysfunction must be identified first. In the four cases presented here, there are four distinct and unique performance paradigms, leading to the suggestion that four different causes and sites of lesion exist. Remediation, obviously, must vary for these specific problems in order to be most effective. Teachers have reported significant improvement in performance when compensatory activities are utilized; these include improved attention resulting from repetition and utilization of other proven intervention strategies. When achievement does not improve after repeated efforts, one can assume either that maturation of the dysfunctional system has not occurred, or that a permanent marginally correctable disability exists. In either case, alternative therapy should be explored.

As clinicians, we suggest that the primary indication for an evaluation by central auditory testing is an apparent hearing loss not explained by peripheral testing. Second, obvious academic failure alone or in association with verbal and/or visual communication disorders requires a thorough assessment of all sensory input modalities. This information is then integrated with the psychometric data and comprehensive psychologic and medical surveys. When correctable disorders, ie, visual or hearing deficits, are identified, appropriate remediation should be initiated.

In summary, the endemic situation of central hearing disability contributes substantially to academic failure. The cause may be genetic or acquired, but regardless, remediation through modified educational strategies and utilization of compensatory listening skills is essential to attainment of maximum academic potential.

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EFFECTS OF TWO BREATH-HOLDING MANEUVERS ON OROPHARYNGEAL SWALLOW

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This study quantified the effects of the supraglottic maneuver (SGM) and super supraglottic maneuver (SSGM) on laryngeal and pharyngeal movements before and during swallow. Simultaneous videofluoroscopic and videoendoscopic examinations of oropharyngeal swallowing were performed in eight healthy volunteers with and without maneuvers. Data analysis compared 1) temporal relationships of oropharyngeal events, 2) airway conditions at the time of selected oropharyngeal events, and 3) biomechanical computer analysis of swallowing events. Using these maneuvers, normal subjects produced earlier cricopharyngeal opening, prolonged pharyngeal swallow, some degree of laryngeal valving before swallow, and change in extent of vertical laryngeal position before swallow. These changes are more successful and maintained longer with the SSGM than the SGM. We concluded that breath-holding maneuvers alter not only airway conditions before swallow but also both the temporal relationships and biomechanical events during oropharyngeal swallow.

KEY WORDS — breath-holding maneuver, laryngeal closure, supraglottic swallow, swallow, videoendoscopy, videofluoroscopy.

INTRODUCTION

Various voluntary swallow maneuvers are known to alter the timing and extent of selected components of oropharyngeal swallowing.[1] Among these voluntary behaviors are two kinds of breath-holding maneuvers: 1) the supraglottic maneuver (SGM), which requires holding the breath lightly, and 2) the super supraglottic maneuver (SSGM), which requires breath holding with increased effort. These maneuvers are designed to minimize aspiration by producing volitional airway protection before, during, and after the swallow. Some authors reported that voluntary breath-holding produced various degrees of laryngeal valving, which increased to arytenoid approximation, true vocal cord (TVC) closure, and anterior arytenoid tilting with an increase in the effort of breath-holding.[2,3] Shaker et al[4] reported that onset of vocal cord closure measured directly from videoendoscopic recordings occurred as the first event during normal swallow, and maximal vocal cord adduction was classified in one of three categories: 1) TVCs were in contact along their entire length, 2) TVCs were in almost complete contact but a small gap remained posteriorly, or 3) TVCs were not in contact with each other. If TVC closure is an important mechanism for preventing the entrance of a bolus into the airway, it is reasonable to identify TVC closure as the time that the TVCs are in complete contact along their entire length. Changes in TVC closure before and during the swallow caused by using voluntary airway closure maneuvers, and the effects of these maneuvers on temporal relationships and biomechanical events during swallow, have never been established.

To quantify the effects of breath-holding maneuvers, it is important to observe both airway conditions and sequential pharyngeal swallow events. Videofluoroscopy is the diagnostic modality that to date allows the greatest visualization of the biomechanics of the oropharyngeal swallow. However, it is difficult to ascertain detailed information on airway closure conditions at the TVC and false vocal cord (FVC) levels with videofluoroscopy.[1] Videoendoscopy may serve as a useful means of illustrating airway conditions directly.[5,6] Perlman and VanDaele[7] reported that videoendoscopic images that were recorded above the epiglottis enable us to identify the temporal information regarding arytenoid and epiglottic behaviors before and partially during the swallow, but they were inadequate to observe TVC behavior during swallow. On the other hand, the endoscopic images that are recorded beneath the epiglottis may provide more detailed information on glottic dynam-

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ics during swallow. Nevertheless, it is not always possible to observe airway conditions, because laryngeal valving and pharyngeal events are obliterated by tissue apposition to the endoscopic lens during laryngeal elevation and pharyngeal contraction. This investigation was designed to examine the effects of these maneuvers (SGM and SSGM) on 1) the temporal characteristics, 2) conditions of the airway entrance, and 3) biomechanical movements of pharyngeal structures in normal subjects before and during the swallow by means of synchronized videofluoroscopy and videoendoscopy.

METHODS

Concurrent videofluoroscopic and videoendoscopic examinations of swallowing were conducted in eight young adult men, between the ages of 20 and 28 years, without a present or past history of swallowing or phonation problems. This study protocol was approved by the Northwestern University Institutional Review Board, and the volunteers gave informed consent before the study. All potential subjects were examined in a pretest to assess tolerance for nasal placement of the endoscope and to determine if visualization of laryngeal structures was adequate.

All subjects were seated in a chair fitted with a headrest and viewed radiographically in the lateral plane throughout the study. A radiopaque marker (US penny = 18 mm) was taped on the skin of the subject's neck to serve as a stationary reference distance marker, against which the movement of structures in the neck could be measured. The lateral fluoroscopic image was displayed on a monitor and recorded on 3/4-in videotape with a video cassette recorder (Sony, VO-5600) at 60 fields per second. Temporal information was encoded at hundredths of a second on each video frame with a counter timer (Thalner Electronics).

Topical anesthesia (2% xylocaine) was applied unilaterally to each subject's nasal cavity, with care taken not to affect pharyngeal mucosa. A 3-mm endoscope (Machida, ENT-3L) was inserted transnasally, and its optical head was advanced to the level of the epiglottis. The endoscopic images from the charge-coupled device camera (Storz, CCII-2) attached to the endoscope were recorded on 1/2-in videotape with a video cassette recorder (Sony, SVO-160). In order to synchronize the videofluoroscopic and videoendoscopic images, temporal information from the fluoroscopy counter timer was recorded on the videoendoscopic 1/2-in videotape at the beginning and end of the videoendoscopic recording session.

The 1/2-in videotape, containing the endoscopic recording corresponding to each swallow recorded with fluoroscopy, was copied onto 3/4-in videotape by means of a Sony U-matic video cassette recorder, model VO-5600. On this occasion, temporal information was encoded at hundredths of a second on each video frame with a counter timer (Thalner Electronics). The two video recordings (videoendoscopy and videofluoroscopy) were able to be exactly time locked to permit temporal synchronization for analysis by comparing and correcting the digital time recorded before and after study.

Each subject completed three swallows of 5 mL liquid barium (E-Z, E-Z EM) using no maneuvers. The liquid barium was placed in the mouth with a syringe and subjects were instructed to hold the barium over the tongue until the swallow command, on which they should swallow it as a single bolus. Then, three swallows each of 5 mL liquid barium were examined as the subjects used two swallow maneuvers: the SGM and the SSGM. Videofluoroscopic and videoendoscopic recordings were initiated before each swallow. Subjects were instructed and trained to continue breathing and then hold their breath lightly before and during swallow (the supraglottic swallow), and to hold their breath hard and bear down before and during swallow (the super supraglottic swallow). After trying the maneuvers several times, they were observed three times and given further instruction as necessary.

Temporal Analysis. Each video recording was analyzed in stop frame and slow motion. Timing of selected oropharyngeal movements from videofluoroscopy was determined by direct reading of the digital time recorded on each video frame. These selected swallow events were 1) oral onset (ie, first backward movement of the bolus), 2) bolus over base of tongue (ie, bolus head passed over the posterior aspect of the ramus of the mandible), 3) onset and termination of hyoid movement, 4) onset and termination of laryngeal movement, 5) onset and termination of arytenoid to epiglottic base closure (laryngeal closure), 6) onset and termination of cricopharyngeal (CP) opening, 7) onset of base of tongue (BOT) movement posteriorly, and 8) onset and termination of BOT to posterior pharyngeal wall (PPW) contact (BOT-PPW contact). The following selected events were defined from videoendoscopic images: 1) onset of arytenoid adduction (ie, the first frame on which initial approximation of arytenoids was observed), 2) onset of arytenoid closure (ie, the first frame on which complete contact of arytenoids along their inner margin was observed), and 3) onset of vocal cord closure (ie, the first frame on which complete

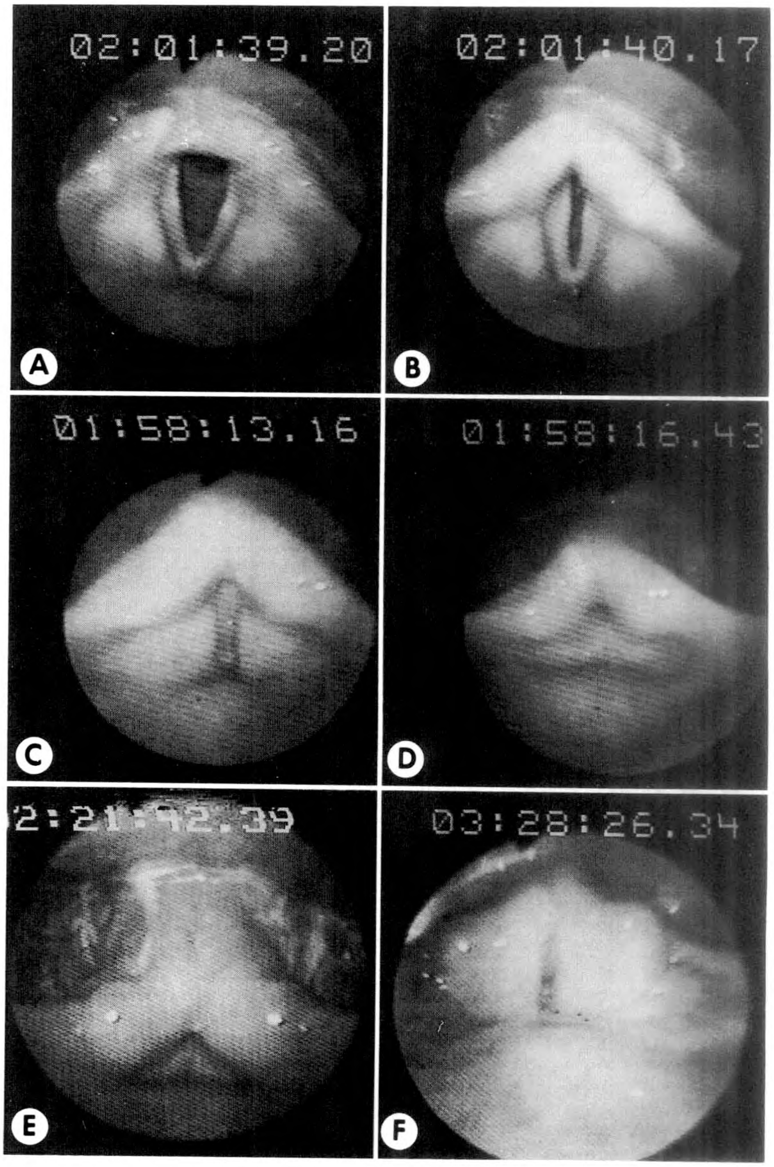

Fig 1. Videoendoscopic images of airway conditions. A) Rest. B) Arytenoid closure. C) True vocal cord closure. D) False vocal cord closure. E) Arytenoid anterior tilting. F) Arytenoid to epiglottic base contact.

contact and closure of the TVCs along their entire length was observed). Timing of each of these selected events was then correlated with the onset of CP opening as time 0.00 s. From these observations, the following measures were made: 1) duration of hyoid movement, 2) duration of laryngeal movement, 3) duration of laryngeal closure, 4) duration of CP opening, and 5) duration of BOT-PPW contact.

Analysis of Airway Conditions. For each swallow, the airway conditions on videoendoscopy were analyzed at the selected oropharyngeal events that were considered as the important points during the swallow. Four selected times of 1) oral onset, 2) bolus over BOT, 3) onset of laryngeal elevation, and 4) return of the larynx to rest were identified from the temporal analysis of videofluoroscopy. The following airway conditions had been observed at these selected times: 1) arytenoid adduction (ie, arytenoid approximation had been already observed), 2) complete arytenoid closure (ie, complete contact of arytenoids along their inner margins had been observed), 3) complete TVC closure (ie, contact and closure of the TVCs along their entire length), 4) FVC closure (contact and closure of the FVCs along their entire length, so that the TVCs were covered by the FVCs), 5) anterior arytenoid tilting (the approximated arytenoid tilted anteriorly over the posterior glottis), and 6) arytenoid to base of epiglottis contact (ie, the arytenoid contacted a bulge at the epiglottic base). (See Fig 1 for examples of these airway conditions.)

Spatial Analysis. Spatial analysis of the videofluoroscopic swallowing sequences was accomplished with an interactive computer program written to enable x-y coordinate determination of selected structures on each video frame. For each swallow, 40 to 60 sequential frames (at one-30th intervals), selected so

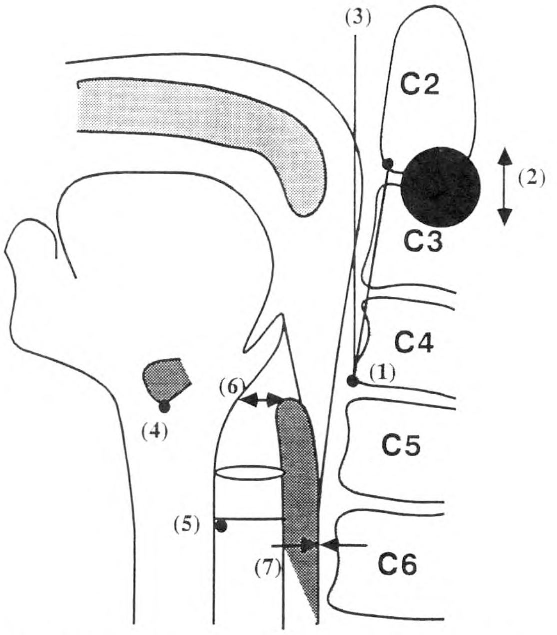

Fig 2. Biomechanical analysis: identification of anatomic points of interest on each frame. 1 — anchor point (C-4 anterior-inferior corner); 2 — radiopaque marker (US penny = 18 mm); 3 — postural angle (angle between anchor point to anterior-inferior corner of second vertebral body and vertical line through anchor point); 4 — anterior-inferior corner of hyoid bone; 5 — anterior-superior corner of subglottic air column; 6 — anterior-superior corner of arytenoid cartilage to posterior surface of epiglottis; 7 — anterior-posterior wall of cricopharyngeal region.

as to encompass critical events in the pharyngeal swallow, were analyzed.[8] For this analysis, the following anatomic points were marked: 1) the anchor point (anterior-inferior corner of the fourth cervical vertebral body), 2) the reference distance (US penny = 18 mm), 3) the postural angle (angle between anchor point to anterior-inferior corner of second vertebral body and vertical line through anchor point), 4) the anterior-inferior corner of the hyoid bone, 5) the anterior-superior corner of the subglottic air column (representing anterior and superior laryngeal position to track laryngeal motion), 6) the width of the laryngeal entrance at the anterior-superior corner of the arytenoid and posterior surface of the epiglottic base opposite the anterior-superior corner of the arytenoid, and 7) the anterior-posterior wall of the CP region. The axial position of the CP region was uniformly marked 1 cm distal to the subglottic air column. These points are illustrated in Fig 2.

After marking the data points on each digitized image, the coordinates were computed. The origin of the coordinate system was referenced to the anchor point (point 0.0 mm). Fluoroscopic magnification was corrected by means of the known size of a US penny (18 mm). Thus, the reference distance allowed us to measure the absolute extent of movement of pharyngeal structures throughout the swallow. The angle of the subject's head position was calculated by measuring the angle of the posterior pharyngeal wall relative to the true vertical of the x-y axis on each video frame (postural angle), so that the angle of the head position was corrected for in the analysis of each swallow. Thus, this analysis yielded the x and y coordinates of each data point on each digitized frame of the swallow sequence corrected for magnification, head tilting, and head movement. Identifying these data points allowed us to track, plot, and measure the vertical and horizontal position of the hyoid and larynx throughout the swallow, as well as closure of the airway at the level of the arytenoid to the epiglottic base.

The following specific events during the swallow were then marked on these plots: 1) oral onset, 2) bolus over BOT, 3) onset of CP opening, and 4) first CP closure. Also, the maximal sagittal diameter of CP opening and the maximal distance of larynx and hyoid movement were measured.

Data across all swallows were pooled, averaged, and expressed as means ± SE unless otherwise specified. Statistical comparisons among each condition were done with two-way analysis of variance (two-way ANOVA) and paired *t*-tests. In all cases, p values of .05 or less were considered statistically significant.

RESULTS

Temporal Relationships. Table 1 summarizes the timing and duration of oropharyngeal events with and without the breath-holding maneuvers. All events are referenced to CP opening as time 0.00 s.

Onset of hyoid movement, laryngeal movement, laryngeal closure, BOT movement, and BOT-PPW contact was significantly later with the SGM than without the maneuver, and with the SSGM than without the maneuver. However, the SGM and SSGM only differ significantly on onset of laryngeal movement. On the other hand, the onset of arytenoid adduction, arytenoid closure, and TVC closure began earlier with breath-holding maneuvers than without the maneuvers, but the difference between the SGM and the SSGM was not significant.

Laryngeal closure and CP opening increased significantly with the maneuvers as compared to the no-maneuver condition. Both measures are also significantly greater with the SSGM than with the SGM ($p < .05$). The duration of hyoid and laryngeal movement was prolonged with breath-holding maneuvers versus no maneuvers, but there were no significant differences between the SGM and the SSGM. On the other hand, no significant difference was seen in the

TABLE 1. EFFECTS OF TWO BREATH-HOLDING MANEUVERS ON TIMING AND DURATION OF OROPHARYNGEAL SWALLOW EVENTS

	Maneuvers			
	None	*SGM*	*SSGM*	*Comparisons†*
Onset of (videofluoroscopy)				
Oral swallow	−0.43 ± 0.02	−0.54 ± 0.06	−0.50 ± 0.03	
Hyoid movement*	−0.21 ± 0.01	−0.18 ± 0.01	−0.15 ± 0.02	1,2
Laryngeal movement*	−0.22 ± 0.01	−0.18 ± 0.01	−0.15 ± 0.02	1,2,3
Laryngeal closure*	0.01 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	1,2
BOT movement*	−0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	1,2
BOT-PPW contact*	0.10 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	1,2
Onset of (videoendoscopy)				
Arytenoid adduction*	−1.32 ± 0.20	−2.62 ± 0.22	−2.29 ± 0.19	1,2
Arytenoid closure*	−0.47 ± 0.07	−1.84 ± 0.27	−1.83 ± 0.16	1,2
TVC closure*	−0.06 ± 0.01	−2.03 ± 0.33	−2.03 ± 0.15	1,2
Duration of (videofluoroscopy)				
Hyoid movement	1.00 ± 0.02	1.13 ± 0.05	1.13 ± 0.05	1,2
Laryngeal movement	1.03 ± 0.02	1.14 ± 0.04	1.14 ± 0.05	1,2
Laryngeal closure*	0.56 ± 0.03	0.60 ± 0.04	0.66 ± 0.04	2,3
CP opening*	0.48 ± 0.01	0.52 ± 0.01	0.57 ± 0.01	1,2,3
BOT-PPW contact	0.44 ± 0.02	0.49 ± 0.03	0.51 ± 0.03	

Values are mean ± SE in seconds. Timing of oropharyngeal events is relative to onset of cricopharyngeal (CP) opening.

BOT — base of tongue, PPW — posterior pharyngeal wall, TVC — true vocal cord.

*$p < .05$ by two-way ANOVA ($df1 = 2$, $df2 = 48$).

†1 — $p < .05$, no maneuver vs supraglottic maneuver (SGM); 2 — $p < .05$, no maneuver vs super supraglottic maneuver (SSGM); 3 — $p < .05$, SGM vs SSGM.

duration of BOT-PPW contact achieved, regardless of maneuvers.

Airway Conditions. On videoendoscopy, information about the laryngeal entrance was clearly observed in almost all trials until the onset of vertical laryngeal movement. After the swallow, information on the laryngeal entrance was visible on only a few trials, because of a coating of barium on the endoscopic lens. The frequency with which each airway condition could be observed endoscopically at four selected pharyngeal events is shown in Table 2.

At oral onset, arytenoid adduction was observed on all trials (100%) with and without breath-holding maneuvers. With breath-holding maneuvers, both complete arytenoid and TVC closure occurred in all trials (100%). Further, the frequency of FVC closure

TABLE 2. FREQUENCY WITH WHICH VARIOUS AIRWAY CONDITIONS WERE OBSERVED ENDOSCOPICALLY DURING NORMAL SWALLOW WITH NO MANEUVERS AND DURING BREATH-HOLDING MANEUVERS AT FOUR SELECTED MOMENTS DURING OROPHARYNGEAL SWALLOW

			Airway Conditions Observed Endoscopically (%)					
	Maneuvers	*N*	*Arytenoid Adduction*	*Arytenoid Closure*	*TVC Closure*	*FVC Closure*	*Anterior Tilting*	*Ary-Epi Contact*
Oral onset	None	24	100	54	13	8	0	0
	SGM	24	100	100	100	17	13	4
	SSGM	23	100	100	100	91	87	46
Bolus over base of tongue	None	18	100	83	23	9	0	0
	SGM	24	100	100	96	13	17	8
	SSGM	23	100	100	100	96	87	48
Onset of vertical laryngeal movement	None	20	100	85	14	0	0	0
	SGM	24	100	100	71	8	22	9
	SSGM	23	100	100	100	91	87	48
Return of larynx to rest	None	9	100	22	11	0	0	0
	SGM	12	100	92	92	33	25	8
	SSGM	6	100	67	67	67	67	67

FVC — false vocal cord, ary-epi — arytenoid to epiglottic base, 0% — events did not occur. See Table 1 for other abbreviations.

TABLE 3. EFFECT OF MANEUVERS ON MOVEMENT OF LARYNX AND HYOID

Selected Swallow Events	*Vertical Movement (mm)*			*Anterior Movement (mm)*		
	No Maneuver	*SGM*	*SSGM*	*No Maneuver*	*SGM*	*SSGM*
Laryngeal movement						
Oral onset	3.2 ± 0.5	4.9 ± 0.7	10.2 ± 1.2*	–0.5 ± 0.4	–0.3 ± 0.4	0.1 ± 0.3
Bolus over BOT	11.3 ± 1.4	11.3 ± 1.1	16.3 ± 1.3*	–1.0 ± 0.4	–0.5 ± 0.3	0.3 ± 0.3
CP opening	24.1 ± 1.1	23.4 ± 1.0	23.0 ± 1.3	3.3 ± 0.7	3.9 ± 0.8	3.3 ± 0.8
CP close	23.0 ± 1.3	23.6 ± 0.5	24.5 ± 1.0	3.7 ± 0.7	3.8 ± 0.6	4.3 ± 0.7
Maximal movement	27.9 ± 1.3	28.0 ± 1.0	29.1 ± 1.3	6.7 ± 0.9	7.2 ± 0.9	7.7 ± 0.9
Hyoid movement						
Oral onset	3.9 ± 0.7	4.1 ± 1.2	2.6 ± 1.7	–0.9 ± 0.3	–1.4 ± 0.3	–1.2 ± 0.4
Bolus over BOT	9.0 ± 1.0	9.7 ± 0.8	8.9 ± 1.1	–0.2 ± 0.8	0.0 ± 0.6	0.3 ± 0.9
CP opening	13.1 ± 1.2	13.8 ± 1.0	12.7 ± 0.9	6.7 ± 0.7	6.9 ± 0.6	4.0 ± 1.0*
CP close	8.7 ± 1.5	9.7 ± 0.8	7.8 ± 1.0	6.6 ± 0.7	7.0 ± 0.6	5.6 ± 0.7
Maximal movement	14.3 ± 1.2	15.6 ± 1.0	15.3 ± 0.9	9.5 ± 0.7	10.1 ± 0.7	9.5 ± 0.7

Values are mean ± SE. See Table 1 for abbreviations.
*p < .05 (no maneuver vs SSGM, and SGM vs SSGM).

and anterior tilting occurred most often with the SSGM.

Complete arytenoid closure and TVC closure were most consistently achieved throughout the visible part of the swallow using the SSGM, whereas the rate of TVC closure using the SGM decreased to 96% at bolus over BOT and to 71% at onset of laryngeal movement in spite of continued arytenoid closure.

Biomechanical Events. The vertical and horizontal movements of the larynx and hyoid at selected oropharyngeal events during swallow with and without maneuvers are shown in Table 3. Significant differences across the maneuvers on vertical laryngeal position occurred at oral onset ($p < .05$). The vertical laryngeal positions at oral onset and at bolus over BOT with the SSGM are significantly higher than with the other conditions ($p < .05$). Similarly, the horizontal hyoid position at CP opening is significantly reduced by using the SSGM. The other positions of the larynx and hyoid at selected swallow points, however, are not modified by the breath-holding maneuvers. The effect of breath-holding maneuvers on laryngeal movement is to increase the vertical laryngeal position before the pharyngeal swallow. Figure 3 depicts the vertical distance between the larynx and hyoid at selected swallow events, which was calculated from the vertical position of the laryngeal air column and hyoid bone. With the breath-holding maneuvers, the distance was significantly reduced at both oral onset and bolus over BOT.

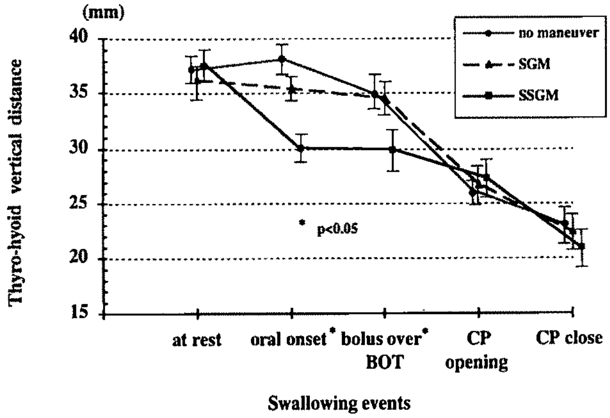

Fig 3. Vertical distance between larynx and hyoid at key oropharyngeal swallow events. Distance is calculated from difference between vertical position of larynx (laryngeal air column) and hyoid (anterior-inferior corner of hyoid bone). Values are mean ± SE (in millimeters) of 8 subjects. With breath-holding maneuvers, vertical distance is significantly reduced at oral onset and when bolus is over base of tongue (BOT). This result indicates that main mechanism of laryngeal elevation is dependent on contraction of thyrohyroid muscle. CP—cricopharyngeus, SGM — supraglottic maneuver, SSGM — super supraglottic maneuver.

With breath-holding maneuvers, the distance between the arytenoid and the epiglottic base was significantly reduced at oral onset and at bolus over BOT ($p < .05$), as shown in Table 4. In addition, this effect is greater during the SSGM than during the SGM ($p < .05$). Figure 4 reveals that maximal CP

TABLE 4. EFFECT OF MANEUVERS ON ARYTENOID TO EPIGLOTTIC BASE DISTANCE

Selected Swallow Events	*No Maneuver*	*SGM*	*SSGM*
Oral onset*	7.4 ± 0.4	4.3 ± 0.3	1.9 ± 0.2
Bolus over BOT*	4.5 ± 0.4	3.7 ± 0.3	1.7 ± 0.3
CP opening	1.4 ± 0.5	1.6 ± 0.4	0.8 ± 0.2
CP close	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0

Values are mean ± SE (mm). See Table 1 for abbreviations.
*p < .05 two-way ANOVA (*df1* = 2, *df2* = 24).

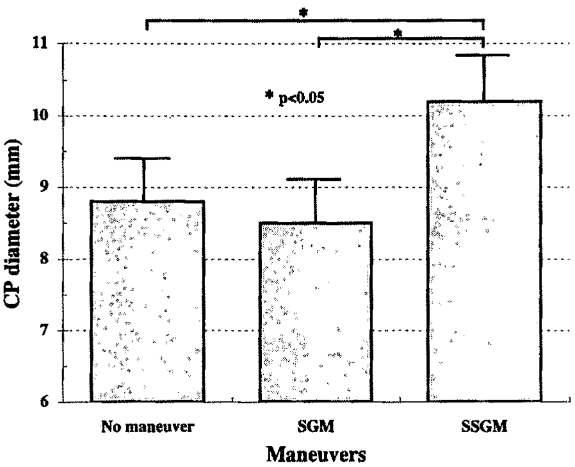

Fig 4. Maximal diameter of cricopharyngeal (CP) opening.

opening is significantly greater during the swallows using the SSGM than during the swallows with the SGM or without a maneuver ($p < .05$).

DISCUSSION

Aspiration associated with swallowing may occur under three circumstances: 1) before the pharyngeal swallow is triggered, when the airway has not elevated or closed; 2) during the swallow, if laryngeal valving is not functioning adequately; and 3) after the swallow, when the larynx lowers and the airway opens for respiration.[9] It is essential to evaluate the effects of breath-holding maneuvers in order to identify the conditions of the airway entrance during the process of swallow. We examined the laryngeal conditions with breath-holding swallows at selected swallowing events using concurrent videoendoscopy and videofluoroscopy. Also, we measured the distance between the arytenoid and the epiglottic base during the swallow, and laryngeal and hyoid position, from videofluoroscopic images to supplement laryngeal information that cannot be visualized by endoscopy during the swallow. Our findings regarding laryngeal conditions at oral onset support previous observations: the arytenoids and TVCs most often move medially as a unit during breath-holding, and the arytenoids tilt anteriorly during effortful breath-holding.[2] These results demonstrate that laryngeal valving with the SSGM has more supplemental function than that with the SGM.

Shaker et al[4] reported that TVC closure during a normal swallow often changed from a position of complete closure when the bolus moved past the BOT to an open position as laryngeal elevation began. In our study, the frequency with which TVC closure occurred with the SGM was 100% at oral onset, and decreased to 71% at onset of laryngeal movement, whereas TVC closure with the SSGM was consistently achieved in 100% of all subjects through onset of laryngeal elevation. Previous investigators reported that the degree of laryngeal valving appears to be related to the effort required, and that it varies in different individuals and trials.[2,3] The fluctuations in laryngeal behavior observed in this study suggest that laryngeal valving with voluntary breath-holding also varies during the process of swallowing, and the SSGM leads to more smoothly continuous laryngeal closure from breath-holding into the swallow. More detailed study of the relationships between glottic behavior and respiratory conditions are needed to elucidate these issues. However, it is obvious that the SSGM is a more effective and successful maneuver than the SGM in protecting the laryngeal inlet and maintaining this protection.

Airway closure during the normal swallow has been characterized as the action of approximating valves progressing inferiorly to superiorly at three different levels: TVCs, FVCs, and aryepiglottic folds, which are augmented by epiglottic retroversion.[10,11] An electromyographic study on temporal behavior of the oropharyngeal muscles during reflex deglutition evoked by superior laryngeal nerve stimulation has shown that the "leading complex," ie, the first detectable action in swallowing, was the submandibular muscles' preceding the activity of the laryngeal intrinsic muscle.[12] On the other hand, some authors have reported that arytenoid approximation is the first event during the swallowing sequence.[13,14] This disagreement about the timing of the arytenoid response seems to depend on the difference in the types of swallowing studied. The former type is the laryngeal closure reflex elicited by superior laryngeal nerve stimulation, whereas the latter is the laryngeal closure in a natural swallow association with respiratory inhibition. Therefore, when the laryngeal closure during swallow is discussed, it is necessary to consider that laryngeal closure may be divided into laryngeal valving with associated respiratory inhibition and laryngeal closure associated with a swallowing sequence that is initiated reflexively.

Logemann et al[14] have reported that the mechanism of laryngeal closure could be divided into two parts: one is under direct neural control by active laryngeal valving, and the other is the biomechanical effect of laryngeal elevation. In our present findings, the onset of arytenoid adduction had already been shown in all subjects invariably at oral onset, even when they were not using a maneuver. Laryngeal valving was enhanced by the instruction to hold the breath. On the other hand, the laryngeal entrance was clearly narrowed at oral onset, but complete contact between the arytenoid and epiglottic base (laryngeal closure as defined on videofluoroscopy) had not occurred at oral onset. Further, the temporal relationship between onset of laryngeal closure and the onset of laryngeal elevation was similar regardless of the use of breath-holding maneuvers. These differences between laryngeal valving as defined on videoendoscopy and laryngeal closure as defined on videofluoroscopy support the observations by Logemann et al that laryngeal closure during swallow is divided into two parts, and indicate that active laryngeal valving can be controlled voluntarily. The effect of breath-holding maneuvers on airway protection is to voluntarily accentuate the laryngeal valving that is usually under neural control, and leads to the second or biomechanical portion of laryngeal closure.

Tight laryngeal valving achieved by effortful breath-holding is probably the result of conjoint contraction of the supraglottic musculature, mainly the thyroarytenoid and thyrohyoid muscles.[15] A previous radiographic study suggested that some degree of laryngeal elevation related to supraglottic closure occurred during the effortful breath-hold.[15] The present spatial analysis of videofluoroscopic images quantified that laryngeal position with effortful breath-holding was significantly elevated, resulting in shortening the distance between the hyoid and the thyroid component. On the other hand, hyoid position was not affected by the breath-holding maneuvers. These findings suggest that the main mechanism of laryngeal elevation associated with breath-holding is apparently dependent on the contraction of the thyrohyoid muscle, which is the main muscular attachment between the hyoid bone and thyroid cartilage. In contrast to these modulations that occur predominantly before the pharyngeal swallow, there were several alterations of temporal relationships and biomechanical events during the pharyngeal swallow. The major modification in the temporal relationships was the forward shift of CP opening, because the other events except for CP opening occur within similar time periods regardless of the breath-holding maneuvers. Overall, the net result with the breath-holding maneuvers can be summarized as 1) earlier CP opening and 2) prolonged pharyngeal swallow.

The mechanical determinants of CP opening are laryngeal elevation and horizontal traction of the hyoid.[16] A study analyzing the mechanism of CP activity during the swallow suggested that sphincter relaxation and its opening were independent.[17] Cricopharyngeal relaxation occurred at roughly the same degree of laryngeal elevation and preceded CP opening, which is associated with a cessation of the tonic activity of the striated muscular structures of the sphincter. In contrast, CP opening results from horizontal traction corresponding to horizontal movement of the hyoid being transmitted to the anterior wall of the CP region via the infrahyoid muscle.[18] We hypothesize that earlier laryngeal elevation associated with the breath-holding maneuvers before the pharyngeal swallow, which produces shortening of the distance between the larynx and hyoid component, plays an important role in the mechanism of earlier CP opening. In other words, the elevation of the larynx before the oropharyngeal swallow may facilitate the transmission of the horizontal jerk of the hyoid bone that contributes to CP opening, so that earlier CP opening is created. The finding that the earliest CP opening was observed with the SSGM, which creates more remarkable elevation of the larynx and reduction in the horizontal position at CP opening, supports this hypothesis. Prolonged CP opening appears to occur because of earlier CP opening and the persistence of laryngeal and hyoid excursion.[19]

Previous studies have reported that the diameter of the CP opening is related to the magnitude of the horizontal excursion of the hyoid bone and the increased intrabolus pressure that is generated by the posterior movement of the BOT.[20] In this study, the maximal diameter of CP opening was significantly increased with the SSGM, despite no change in maximal excursion of the hyoid and larynx. There are several possibilities to explain the increased diameter of CP opening. One is the reconfigurative effect that occurs as a result of earlier laryngeal elevation, which may facilitate the CP opening and produce the same effect as increased hyoid excursion. Our finding that the CP opening occurred at less than the maximal horizontal point of the hyoid may support this possibility. The other possible mechanism is the augmentation of tongue movement with effortful breath-holding, which may produce more vigorous bolus propulsion and increase intrabolus pressure.[20] Further studies, including manometric evaluations, are needed to elucidate this mechanism.

We conclude from this study that the effect of breath-holding maneuvers in normal subjects is to increase laryngeal valving and elevate the laryngeal position before the pharyngeal swallow. These alterations may secondarily produce temporal changes during the pharyngeal swallow. However, the effects of these maneuvers may be different in dysphagic patients who have impaired airway closure. Nevertheless, identification of the effects on normal oropharyngeal swallow events caused by breath-holding maneuvers helps to determine indications for use of these maneuvers in dysphagic patients.

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ONTOGENETIC EXPRESSION OF SPOT 35 PROTEIN (CALBINDIN-D28K) IN HUMAN OLFACTORY RECEPTOR NEURONS AND ITS DECREASE IN ALZHEIMER'S DISEASE PATIENTS

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Expression of a calcium-binding protein, spot 35 protein (S-35, calbindin-D28k), was investigated immunohistochemically in the human olfactory mucosa of patients who ranged in age from 16 weeks of fetal development to 98 years old, including some with Alzheimer's disease (AD). S-35 immunoreactivity was observed clearly in olfactory receptor neurons (ORNs) and olfactory nerve bundles that were identified previously with antibodies to olfactory marker protein (OMP) and neuron-specific enolase (NSE). Throughout all ages, the mean number of ORNs immunoreactive for OMP did not change significantly, whereas the mean number of NSE- and S-35–immunoreactive ORNs declined markedly in the postnatal infant, young, and old patients when compared with that of the prenatal fetuses. S-35–immunoreactive ORNs decreased significantly in AD patients when compared with AD control patients. These results indicate that ORNs in humans express S-35 and that there is an age-related trend in the expression of S-35. Furthermore, the marked decrease of S-35 expression in ORNs of AD patients suggests that cell excitability associated with calcium ions and cell protective function against overload of intracellular calcium ions decline in these patients.

KEY WORDS — Alzheimer's disease, calbindin-D28k, human olfactory mucosa, immunohistochemistry, neuron-specific enolase, olfactory marker protein, ontogeny, spot 35 protein.

INTRODUCTION

Changes in calcium ion (Ca^{2+}) levels are associated with several processes within neurons. These include, in some neurons, the transduction of stimuli, shaping of the action potential, transmitter release, and synapse modification.[1] Many cells contain cytosolic calcium-binding proteins that either modulate or mediate the actions of Ca^{2+}.[2] These proteins have been proposed to play several important roles in neurons. The most important role is related to neuronal cell excitability coupled with Ca^{2+} activity.[3] Recently, an additional role was suggested: calcium-binding proteins may protect neurons from an overload of intracellular Ca^{2+} that is caused by neurotoxic glutamate, ischemia, or seizure-induced neuronal damage.[4-6] In the central and peripheral nervous systems of mammals, including humans, many types of calcium-binding proteins have been identified, and several, such as parvalbumin, calbindin-D28k, calretinin, calcineurin, and S-100 protein, have been found in high concentrations.[2,7] In addition, these proteins were reported to decrease in several pathologic conditions. For example, in Alzheimer's disease (AD) and Down syndrome, it has been shown that the expression of several types of calcium-binding proteins decreased in several types of brain neuronal cells.[8-10] Moreover, in olfactory receptor neurons (ORNs) and olfactory nerve bundles, the expression of a calcium-binding protein, spot 35 protein (S-35), decreased with aging in animals.[11]

Spot 35 protein is a soluble protein that was first identified by Yoshida and Takahashi[12] in rat cerebellum using two-dimensional gel electrophoresis. Yamakuni et al[13] isolated this protein and made an antibody against it. This protein has Ca^{2+}-binding activity that regulates the excitability of the Purkinje cell.[14,15] Subsequently, S-35 was recognized as being the same calcium-binding protein as calbindin-D28k. Their amino acid sequences are 99.6% similar.[16-18] In the animal olfactory mucosa, expression of S-35 (calbindin-D28k) and calretinin was identified in ORNs and "microvillar cells" by immunohistochemistry.[11,19-21] Furthermore, in the human olfactory mucosa from one patient, we observed weak S-35 immunoreactivity in the epithelium, although the cell type was not clear.[21] However, in these previous reports a consistent localization and expression pattern was not apparent.

The first purpose of our present study was to

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TABLE 1. SOURCES OF HUMAN OLFACTORY MUCOSA

Patients	*Age*	*Sex*	*Cause of Death*	*PMI (h)*	*FT (h)*
Normal patients					
1	16 wk fetus	M	Spontaneous abortion	6.25	2
2	26 wk fetus	F	Spontaneous abortion	8	2
3	10 wk infant	M	Congenital heart disease	6.5	18
4	24 y	M	Gunshot wound (chest)	22	3
5	45 y	F	Lung cancer	11.5	2
6	47 y	F	Gunshot wound (chest)	17	1
7	65 y	M	Renal failure	5.5	2
8	90 y	M	Hypertension	19.5	2
			Mean ± SE	12.0 ± 2.3	4.0 ± 2.0
ADCs					
1	77 y	M	Pancreatic cancer	3	3
2	76 y	M	Metastatic cancer	4	1
3	82 y	F	Cardiac failure	5	2
4	78 y	F	Myocardial infarction	3	2
5	98 y	M	Myocardial infarction	2.75	2
6	69 y	M	Multiple myeloma	3.3	2
Mean ± SE	80.4 ± 4.0			3.5 ± 0.4*	2.0 ± 0.3
ADs					
1	72 y	F	Upper GI tract hemorrhage	3	5
2	52 y	M	Pneumonia	3.8	4
3	72 y	M	Lung emphysema	3.8	5
4	85 y	F	Cardiac failure	1.5	2
5	74 y	M	Cardiac failure	12.5	1.5
6	70 y	M	Pneumonia	2.5	2
7	72 y	M	Unknown	4.3	2
8	90 y	M	Pneumonia	3	2
9	87 y	M	Cardiorespiratory arrest	4.75	2
Mean ± SE	75.0 ± 3.9			4.4 ± 1.1*	2.8 ± 0.5

PMI — postmortem interval, FT — fixation time, ADC — Alzheimer's disease control patients, AD — Alzheimer's disease patients, GI — gastrointestinal.

*$p < .01$ in comparison to normal patients.

examine the presence of S-35–immunoreactive cells in the human olfactory mucosa, identified by the presence of olfactory marker protein (OMP)– and neuron-specific enolase (NSE)–immunoreactive ORNs. The second purpose was to investigate the ontogenetic expression of S-35 in different-age patients, in relationship to OMP and NSE expression. Last was to investigate the expression of S-35 in the olfactory mucosa of patients with AD and their age-matched controls (Alzheimer's disease controls; ADCs).

MATERIALS AND METHODS

Materials. Human olfactory mucosa was obtained at autopsy from 23 patients in age groups ranging from 16 weeks of fetal development to 98 years old, including 8 normal patients (divided into prenatal fetus, postnatal infant, and young and old groups), 6 ADCs, and 9 AD patients (both AD and senile dementia of Alzheimer's type were included). According to the AD research project conducted at the Alzheimer's Disease Research Center of the Sanders-Brown Center on Aging, University of Kentucky, ADC and AD patients were examined with the same neuropsychological testing, and the ADC patients were confirmed not to have dementia. All specimens were obtained at the time of autopsy from the olfactory cleft and fixed immediately with ice-cold Zamboni's fluid. Then they were cryoprotected with graded phosphate-buffered sucrose solution, embedded in OCT compound (Tissue-Tek, Miles Laboratories, Elkhart, Ind), and rapidly frozen on dry ice–ethanol. Cryostat sections were cut at 15 µm thickness. Although the mean postmortem interval in the normal patients was statistically longer than that of the ADC and AD groups, the postmortem stability of calbindin-D28k was previously reported in the human central nervous system and it was confirmed that there was no significant correlation between postmortem delay and the number of calbindin-D28k–

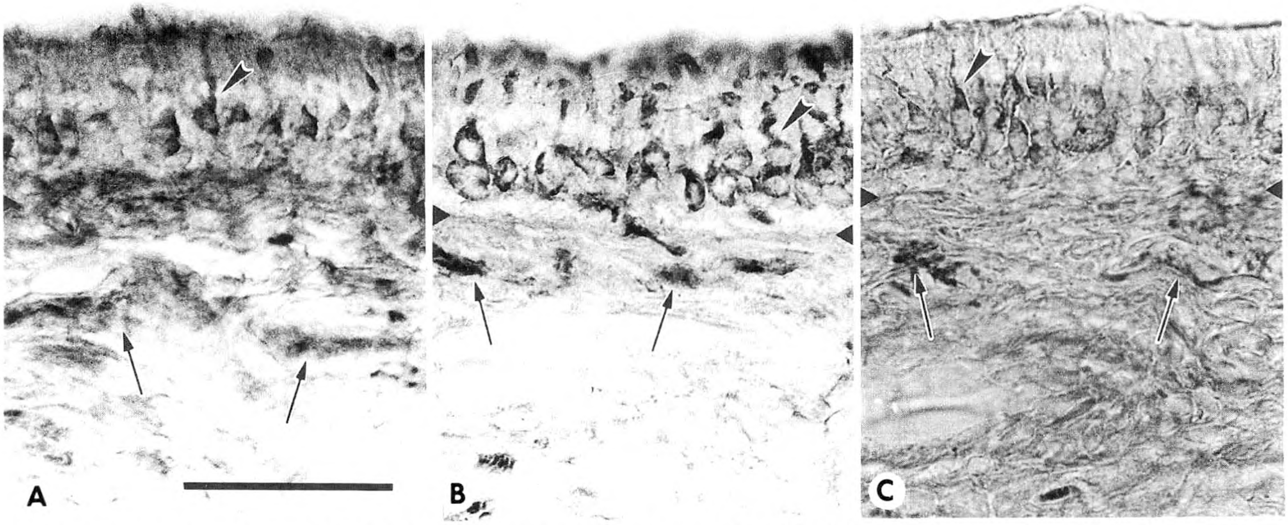

Fig 1. Immunohistochemical identification of olfactory receptor neurons (ORNs) with antibodies to A) olfactory marker protein (OMP), B) neuron-specific enolase (NSE), and C) spot 35 protein (S-35) in olfactory mucosa (normal patient 6, 47 years old). S-35 expression identifies ORNs (arrowhead on C) that are also immunoreactive to anti-OMP (A) and NSE (B) antisera. Cytosolic immunoreactivity is distributed throughout apical olfactory knobs, dendrites, and perikarya of bipolar ORNs in epithelium, and olfactory nerve bundles (arrows in A-C) in lamina propria. Bar — 50 μm, triangles — basement membrane.

immunoreactive neurons.[9] It therefore seems likely that the immunoreactive material detected in the normal patient group was authentic S-35 (calbindin-D28k). Detailed data on the patients are shown in Table 1.

Immunohistochemical Methods. The standard streptavidin-biotin-peroxidase complex method with the appropriate species-specific Histostain-SP Kits (Zymed Laboratories Inc, South San Francisco, Calif) was used for immunostaining. Briefly, sections were treated with 50% methanol containing 5% hydrogen peroxide and incubated with 2% bovine serum albumin for 30 minutes each, and then incubated with one of the following primary antibodies at room temperature for 1 hour: rabbit anti–S-35 (1:600), NSE (1:6,000), or goat anti-OMP (1:10,000). Polyclonal anti–S-35 antiserum was made against S-35 that was derived from rat cerebellum, and was a gift from Dr R. Kuwano (Research Laboratory for Molecular Genetics, Niigata University, Japan). Its specificity was previously reported.[13] Olfactory marker protein antiserum was supplied by Dr F. L. Margolis (Roche Institute of Molecular Biology, Nutley, NJ). Polyclonal NSE antiserum was obtained from Polysciences Inc (Warrington, Pa). The sections were further incubated in biotinylated secondary antibodies and peroxidase-conjugated streptavidin for 10 minutes each. Reacted sections were exposed to aminoethylcarbazole and hydrogen peroxide to develop the stain.

As a positive tissue control for S-35, rat olfactory bulb was used, because the periglomerular cells were reported to be S-35–immunoreactive.[20] As a negative control, sections were processed by the omission of the primary antibodies. No specific labeling was observed in any negative control sections.

Numeric Analysis. For numeric analysis, we selected 4 to 6 fields per specimen of each patient. In each field, the number of neurons whose perikarya showed well-defined immunoreactivity was counted in 200 μm epithelial length. Statistical analyses comparing the numbers of OMP-, NSE-, and S-35–immunoreactive ORNs of all groups were performed with the two-sided Mann-Whitney U test. Furthermore, the ratios of the numbers of OMP-, NSE-, and S-35–immunoreactive ORNs were compared among groups.

RESULTS

S-35 Expression and Localization. The olfactory mucosa from all patients listed in Table 1 was identified to contain bipolar ORNs, which were immunoreactive to both anti-OMP (Fig 1A) and NSE (Fig 1B) antisera. In the sections adjacent to those containing OMP- and NSE-immunoreactive neurons, bipolar ORNs also clearly exhibited S-35 immunoreactivity. Spot 35 protein expression was localized in the cytosol of apical olfactory knobs, dendrites, and perikarya. Furthermore, S-35–labeled nerve bundles containing the axons of ORNs were observed in the lamina propria (Fig 1C) that were also immunoreactive to anti-OMP and NSE antisera. Spot 35 protein–immunoreactive ORNs were observed in the sections

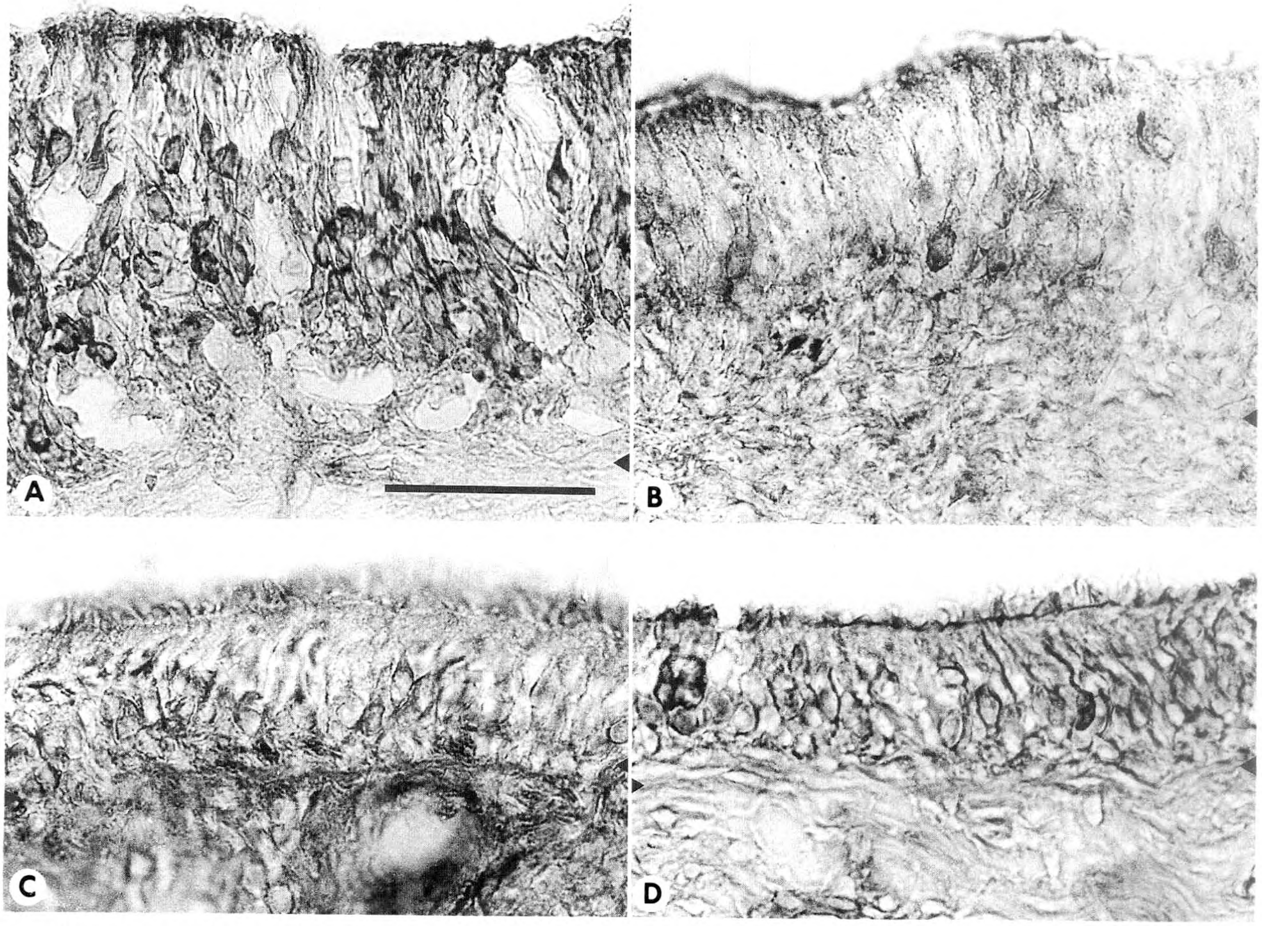

Fig 2. Ontogenetic expression of S-35. Number of S-35–immunoreactive ORNs shows marked decrease between A) prenatal and B-D) postnatal stages. In postnatal stages, number of S-35–immunoreactive ORNs is similar among patients. Bar — 50 µm, triangles — basement membrane. A) Patient 1, 16-week fetus. B) Patient 3, 10-week infant. C) Patient 4, 24 years old. D) Patient 7, 65 years old.

from all ages of patients that we examined.

Age-Related Trend of S-35 Expression and Relationship Among OMP, NSE, and S-35 as Expressed in Normal Patients. There were characteristic differences in the number of S-35–immunoreactive ORNs among the specimens from different categories of patients. In general, the number of immunoreactive neurons was greatest in the tissues from the prenatal fetus group (16 weeks, 26 weeks) and was noticeably less in the tissues from the postnatal infant (10 weeks), young (24 years, 45 years, and 47 years), and old (65 years and 90 years) groups. In all postnatal age groups, the number of S-35–immunoreactive ORNs was similar, although the thickness of the olfactory epithelium exhibited a gradual decrease in association with age (Fig 2). In addition, our statistical analysis showed that there was a clear age-related trend in the mean number of OMP-, NSE-, and S-35–immunoreactive ORNs per unit length of the olfactory epithelium. In the prenatal fetus group, numerous ORNs displayed immunoreactivity for S-35, and the mean number of S-35–immunoreactive neurons was similar to that of NSE. In contrast, the mean number of OMP-immunoreactive ORNs was less than those of NSE and S-35. In the postnatal infant group, the mean number of ORNs showing NSE and S-35 immunoreactivity significantly decreased when compared with that of the prenatal fetus group (Table 2, $p < .001$ and .01, respectively). In the young and old groups, the mean number of NSE- and S-35–immunoreactive neurons also declined when compared with that of the prenatal fetus group (Table 2). Statistical analysis showed that a significant difference in the mean number of NSE-immunoreactive neurons existed among the prenatal fetus group and the young ($p < .001$) and old groups ($p < .01$). The mean number of S-35–immunoreactive neurons in the old group also significantly decreased when compared with that in the prenatal fetus group ($p < .001$). However, the mean number of OMP-immunoreactive neurons was similar and did not exhibit statistically significant changes throughout the age groups, although the mean number of immunoreactive neurons was slightly

TABLE 2. IMMUNOREACTIVE OLFACTORY RECEPTOR NEURONS IN OLFACTORY EPITHELIUM

Patient	*OMP*	*NSE*	*S-35*	*Ratio* *OMP:NSE:S-35*
Prenatal fetus	22.1 ± 4.8 (8)	54.9 ± 2.8 (11)	57.5 ± 5.2 (8)	1 : 2.5 : 2.6
Postnatal infant	21.5 ± 2.6 (4)	22.5 ± 2.6 (4)*	20.8 ± 1.7 (4)†	1 : 1.0 : 1.0
Young	15.8 ± 1.1 (12)	28.4 ± 2.3 (12)*	22.8 ± 2.4 (12)	1 : 1.8 : 1.4
Old	21.9 ± 4.6 (8)	30.4 ± 3.0 (8)†	17.4 ± 2.3 (8)*	1 : 1.4 : 0.8
ADC	20.0 ± 2.0 (27)	30.7 ± 2.3 (35)	19.6 ± 1.1 (36)	1 : 1.5 : 1.0
AD	16.7 ± 0.9 (37)	28.8 ± 1.0 (52)	8.4 ± 0.9 (51)§	1 : 1.7 : 0.5

Number of immunoreactive neurons was counted in 200 μm epithelial length in 4 to 6 fields per each specimen from 2 prenatal fetuses (16 wk, 26 wk), 1 postnatal infant (10 wk), 3 young (24 y, 45 y, 47 y) and 2 old (65 y, 90 y) patients, 6 ADC patients, and 9 AD patients, then averaged. Numbers in parentheses show total fields that were counted.

OMP — olfactory marker protein, NSE — neuron-specific enolase, S-35 — spot 35 protein. Other abbreviations as in Table 1.

*$p < .001$ compared to prenatal fetus; two-sided Mann-Whitney U test.

†$p < .01$ compared to prenatal fetus; two-sided Mann-Whitney U test.

§$p < .001$ compared to ADC; two-sided Mann-Whitney U test.

decreased in the young group (Table 2).

There were characteristic changes in the ratio of the numbers of the OMP-, NSE-, and S-35–immunoreactive ORNs in each age group. In the prenatal fetus group, the numbers of NSE- and S-35–immunoreactive neurons were similar and 2.5 times greater than the number of OMP-immunoreactive ORNs. In the postnatal infant group, the ratio was 1:1:1. In the young group, the ratio of NSE- to S-35–immunoreactive neurons increased again. In the old group, the number of S-35–immunoreactive neurons declined slightly and was similar to that of OMP-immunoreactive neurons (Table 2).

Differences Between ADC and AD Patients. Expression of S-35 was also shown clearly and intensely in the olfactory mucosa in all specimens of the ADC group. In these patients, the mean number of ORNs expressing S-35 was similar to that of ORNs expressing OMP, whereas the mean number of NSE-immunoreactive neurons was 1.5 times greater than that of S-35– and OMP-immunoreactive neurons (Table 2 and Fig 3A,B). In contrast, S-35 expression was notably reduced in the AD group; that is, the mean number of S-35–immunoreactive ORNs in the AD group was significantly less than that in the ADC group (Table 2, $p < .001$), although the mean numbers of OMP- and NSE-immunoreactive neurons were not significantly different in these two groups. Only a few ORNs were reactive to the anti–S-35 antiserum as compared to NSE antiserum (Fig 3C,D).

DISCUSSION

The results of our study have demonstrated that 1) ORNs express S-35; 2) there is an age-related trend in the expression of S-35 immunoreactivity in ORNs; that is, the number of S-35–immunoreactive ORNs is greatest in prenatal fetuses and decreases significantly after birth; 3) the ratio of the numbers of OMP-, NSE-, and S-35–immunoreactive ORNs exhibits characteristic changes within each age group; and 4) there is a marked decrease in the expression of S-35 in ORNs in AD patients when compared with that of ADC patients.

Significance of S-35 Expression in ORNs. Expression of S-35 (calbindin-D28k) in the olfactory mucosa has been reported in animals and humans.[11,19-21] In animals, its immunoreactivity was localized in ORNs of rats[11] and mice,[19] and present in the "microvillar cells" that were identified as the fourth cell type in the olfactory epithelium of guinea pigs.[20] However, in human olfactory epithelium embedded in paraffin, we could observe only weak S-35 immunoreactivity in a flask-shaped cell that was similar in shape to those seen in guinea pigs.[21] In the present study, using the olfactory mucosa from 23 patients, we demonstrated that one of the buffer-type calcium-binding proteins, S-35, was present in ORNs that were identified unambiguously by OMP and NSE antisera. Our new result indicates that S-35 may be a useful marker for examining the localization and ontogenetic expression of calcium-binding proteins in the human olfactory mucosa.

In the olfactory transduction, Ca^{2+} is one of the ions that enters ORNs through cation-permeable membrane channels.[22,23] Increase of intracellular Ca^{2+} blocks the cyclic nucleotide–gated channels[24-26] and activates Ca^{2+}-dependent potassium ion channels[27] of ORNs in animals. In humans, a recent study has also demonstrated that isolated ORNs respond to odor stimuli with an increase of intracellular Ca^{2+}.[28] Since S-35 has a Ca^{2+}-buffering capacity and is present in human ORNs, it is suggested that S-35 may have a role in regulating Ca^{2+} activity associated with ion channels in human olfactory transduction.

Age-Related Trend in S-35 Expression. The age-related trend of S-35 expression in this study, that is,

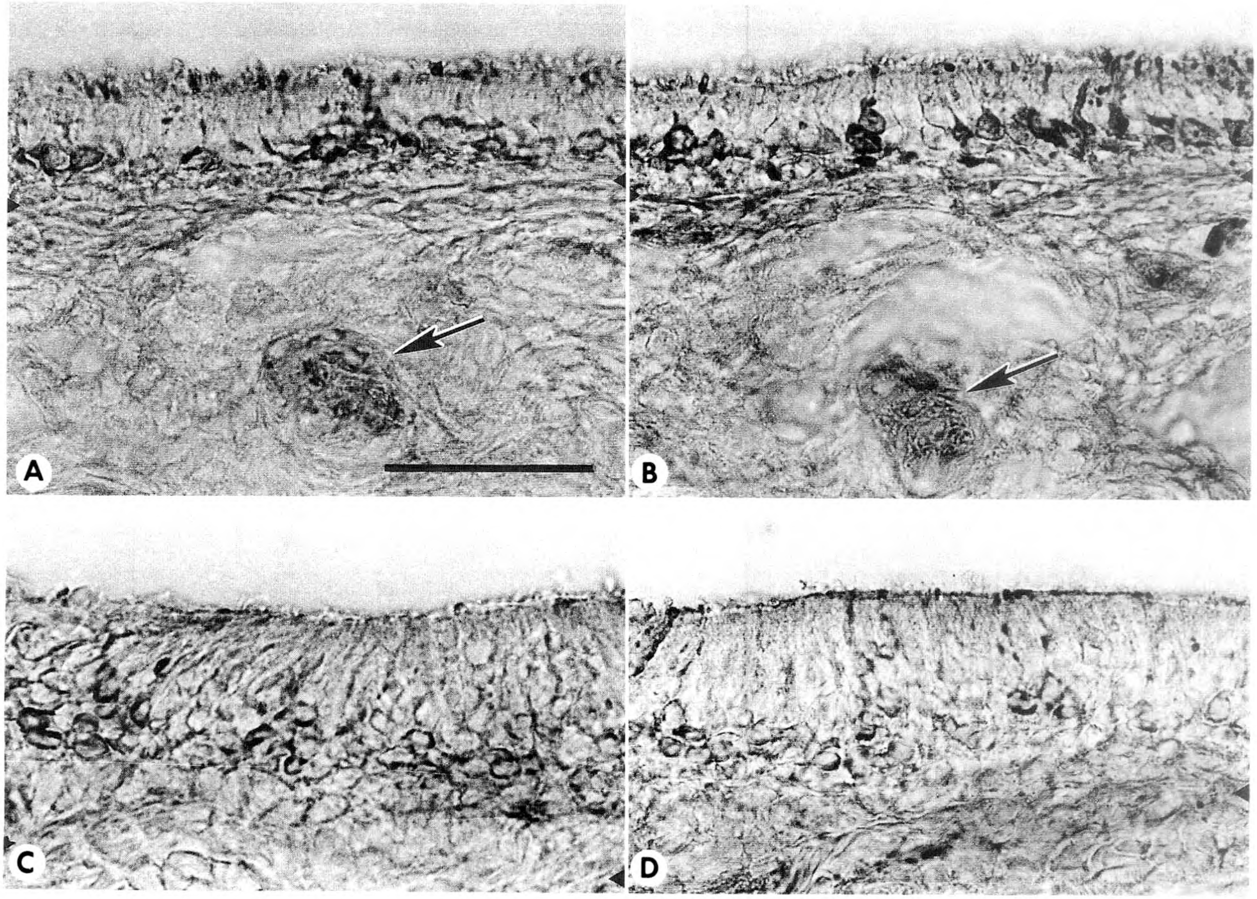

Fig 3. NSE and S-35 expression in olfactory mucosa of A,B) control and C,D) Alzheimer's disease patients. In control patient (control patient 2), majority of NSE-immunoreactive ORNs (A) also expressed S-35 (B). In Alzheimer's disease patient (Alzheimer's patient 4), number of S-35–immunoreactive ORNs (D) decreased markedly when compared with number of NSE-immunoreactive neurons (C; see Table 3 also). Bar—50 µm, triangles—basement membrane, arrows—olfactory nerve bundles in lamina propria.

the marked difference between the prenatal fetus group and the postnatal age groups, suggests that expression of S-35 increases in the early stage of gestation and decreases markedly shortly after birth. In a previous study using various ages of rats (Abe et al[11]), the expression of S-35 in ORNs quickly declined at postnatal stages and disappeared in old age. Our data obtained from the human olfactory mucosa are somewhat similar to the rat data, in that decrease of S-35 quickly occurs after birth, although it does not disappear in old patients.

Ratio of OMP, NSE, and S-35. The mean number of OMP-immunoreactive ORNs was less than that of NSE in all age groups in our study, and the ratio of OMP- to NSE-immunoreactive neurons showed characteristic changes in each age group of normal patients. Getchell and Getchell[29] recently reported a similar age-related trend in OMP and NSE expression in human ORNs. As reported in animal experiments, it was proposed that OMP is expressed only in mature ORNs that have synaptic contacts with second-order neurons in the olfactory bulb.[30,31] The ratio of OMP- to NSE-immunoreactive ORNs may be affected by the extent of neurogenesis at each age. In addition, our study demonstrates that the ratios of S-35– to OMP-immunoreactive ORNs in the postnatal infant, young, and old groups were similar. This result strongly suggests that after birth, S-35 is expressed in mature ORNs that probably express OMP as well. In contrast, the numbers of NSE- and S-35–immunoreactive ORNs were similar and 2.5 times greater than that of OMP-immunoreactive neurons in the prenatal fetus group. Thus, we suggest that the majority of NSE-expressing ORNs that represent both mature and immature neurons also express S-35 in the prenatal fetus.

Difference in S-35 Expression in ADC and AD Patients. Between ADC and AD patients, there was no statistical difference in the mean number of NSE- and OMP-immunoreactive ORNs, although there was a trend toward a decreased number within the limit of our samples. This suggests that the olfactory mucosa of AD patients contains mature and immature ORNs similar in number to those of ADC patients. In contrast, the mean number of ORNs expressing S-35 in AD patients was significantly less when compared with ADC patients, and the number of S-35–expressing neurons was markedly decreased when compared with those of OMP- and NSE-immunoreactive neurons. These results indicate that in the olfactory mucosa of AD patients there is a selective reduction in the expression of S-35.

To date, there have been two conflicting hypotheses: whether the numbers of calbindin-D28k–expressing neurons are reduced, or whether they remain in the brain tissue of patients with neurodegenerative disorders including AD. Several authors reported a decrease in the levels of calbindin-D28k and its gene expression in the brain tissue of patients with AD.[9,32] However, other authors reported that the expression of calbindin-D28k messenger RNA and the level of the protein were different from area to area,[33] or that the levels did not change.[34] Our study demonstrates that only S-35 (calbindin-D28k)–expressing ORNs decrease in number in AD patients, and therefore supports the first hypothesis, although our result is limited only to ORNs. Moreover, neural excitability, which is affected by Ca^{2+} entry, may decrease in ORNs in AD patients, because it was reported in rats that the neurons that were depleted of their calbindin-D28k content had a lower whole-cell Ca^{2+} current than those containing this protein.[35] Furthermore, cell protective function against an overload of intracellular Ca^{2+} may decrease in ORNs in AD. These changes may be the causes of the olfactory disturbance that has been reported by many authors[36-39] to occur in the early stage of AD.

S-35 as Tool for Definitive Diagnosis of AD. Recently, an olfactory mucosal biopsy has been developed to diagnose AD definitively.[40-42] It is known that several kinds of τ proteins and ubiquitin are preferentially accumulated in biopsy specimens of olfactory mucosa of AD patients,[41,42] as well as in autopsy specimens.[43,44] We stress that S-35, which exhibited a specific decrease in AD patients in this study, may become another useful marker for definitive diagnosis of AD, in combination with other markers such as τ and ubiquitin.[40,41]

In summary, S-35 is expressed in human ORNs, and its expression decreases after birth. Furthermore, the number of ORNs expressing S-35 declines significantly in AD patients. Therefore, S-35 may be of some value in the characterization of AD by means of olfactory mucosal biopsy in patients.

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EFFECTS OF PLATELET ACTIVATING FACTOR ON MUCOCILIARY CLEARANCE OF THE EUSTACHIAN TUBE

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The effects of platelet activating factor (PAF) on mucociliary clearance of the eustachian tube were investigated both in vitro and in vivo. Normal ciliated epithelium was obtained from the eustachian tube of guinea pigs and incubated with PAF at concentrations ranging from 10^{-10} to 10^{-6} mol/L. Ciliary activity was observed under an inverted microscope and quantified photoelectrically. The PAF dose-dependently inhibited ciliary activity. One milliliter each of 10^{-5} mol/L PAF, 10^{-5} mol/L prostaglandin E2 (PGE2), 10^{-5} mol/L PAF and PGE2, or the control solution (0.1 v/v% methanol–phosphate-buffered saline) was directly injected into the tympanic bullae of anesthetized chinchillas. The middle ear was examined by otomicroscopy, tympanometry, and auditory brain stem response in relation to time. The PAF delayed middle ear clearance, and the PGE2 augmented its delay. These findings suggest that PAF inhibits mucociliary clearance of the eustachian tube from the middle ear, and that PGE2 plays an important role in the augmentation of inflammatory disorders.

KEY WORDS — **middle ear clearance, mucociliary transport, otitis media with effusion, platelet activating factor, tubal mucosa.**

INTRODUCTION

Platelet activating factor (PAF), in addition to leukotrienes (LTs) and prostaglandins (PGs), is one of the most important lipid mediators in inflammatory disorders. Platelet activating factor shows various biologic effects such as activation of inflammatory cells,[1,2] elevation of vascular permeability,[3] and induction of hypersecretion by submucosal glands.[4] It is well known that PAF is generated de novo by neutrophils,[5] macrophages,[6] and other inflammatory cells. In the effusion of patients with otitis media with effusion (OME), both neutrophils and macrophages,[7,8] and also many mediators such as histamine,[9] bradykinin,[10] LTs, and PGs,[11] have been detected. In addition, Cauwenberge and Bernstein[10] reported the presence of PAF in the middle ear effusion of such patients at concentrations of 10^{-9} to 10^{-8} mol/L. Platelet activating factor induces ciliary dysfunction and epithelial damage of human paranasal sinus mucosa in vitro; the threshold concentration of PAF that can affect ciliated epithelium and induce ciliary inhibition is approximately 10^{-10} mol/L.[12] Consequently, it is possible that PAF inhibits mucociliary clearance of the eustachian tube and plays an important role in the accumulation of effusion in the middle ear. To investigate the pathogenesis of OME, it is necessary to determine whether PAF inhibits the tubal transport system. However, there have been few reports concerning the role of PAF in the middle ear. Furthermore, these various kinds of mediators detected in the middle ear effusion of OME patients may interact when mixed. Prostaglandin E2 is a common mediator of inflammation, inducing vasodilatation.[13] Prostaglandin E2 has been reported to promote ciliary activity of tracheal[14] and eustachian tube[15] mucosa in vitro. Prostaglandins such as PGE1 and PGE2 accelerate ciliary beating, while PAF does not. There have been conflicting reports concerning whether LTC4 and LTD4 also accelerate ciliary beating.[14-19] Prostaglandin E2 certainly differs from PAF in its effect on ciliary activity, and the effect of the interaction between PAF and PGE2 in the middle ear is of interest. In the case of LTC4, Jung et al[20] also reported that PGE2 augmented the effect of LTC4 in inducing middle ear inflammation and effusion. In the present report, we describe the effects of PAF on mucociliary clearance of the eustachian tube both in vitro and in vivo, and additionally, the interactive effects of PGE2 and PAF are discussed.

MATERIALS AND METHODS

Platelet Activating Factor and Prostaglandin E2. Platelet activating factor 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine (Bachem Feinchemikalien AG, Bubendorf, Switzerland) and PGE2 (Funakoshi Co, Tokyo, Japan) were used. The PAF was dissolved in methanol at a concentration of 10^{-2} mol/L and then diluted to final concentrations of between

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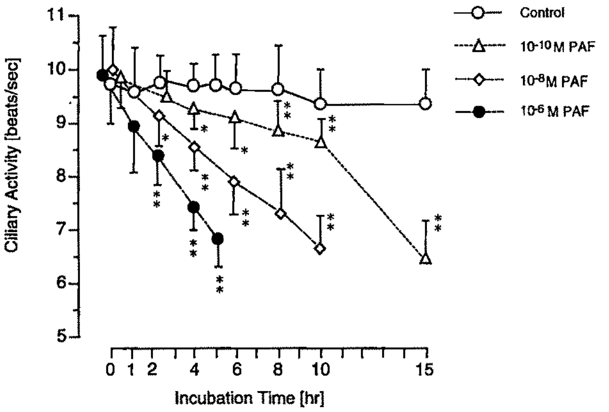


Fig 1. Effects of platelet activating factor (PAF) on ciliary activity of eustachian tube mucosa of guinea pigs at concentrations ranging from 10^{-10} to 10^{-6} mol/L in vitro. Ten ciliated cells were observed in each experiment. Values are expressed as mean ± SD. * — $p < .05$ versus control, ** — $p < .01$ versus control.

10^{-5} and 10^{-10} mol/L before use. The PGE2 was dissolved in ethanol also at a concentration of 10^{-2} mol/L and diluted to the final concentration of 10^{-5} mol/L. Each test solution was diluted with Roswell Park Memorial Institute (RPMI) 1640 solution for in vitro study or with phosphate-buffered saline (PBS) for in vivo study. The control solution without mediators was prepared in the same manner as the experimental solution. Consequently, we used 0.01 v/v% methanol–RPMI 1640 and 0.1 v/v% methanol-PBS solution for in vitro and in vivo study, respectively.

Observation of Ciliary Activity In Vitro. Healthy guinea pigs with normal Preyer's reflexes (280 to 350 g) were used. The animals were anesthetized by intraperitoneal injection of sodium pentobarbital solution (50 mg/kg) and then painlessly sacrificed. Normal mucosa was carefully obtained from the tympanic orifice and the osseous portion of the eustachian tube. The mucosal specimens were rinsed to remove mucus and blood cells, cut into pieces, and transferred to culture dishes containing RPMI 1640 solution supplemented with 10% fetal calf serum, and ciliated epithelium was incubated in the form of tissue culture before exposure to each test solution. The experiments were performed at 37°C under an inverted microscope equipped with a thermoregulator and a humidified carbon dioxide chamber (Olympus Co, Tokyo, Japan). Ciliary activity of ciliated cells was observed and quantified photoelectrically. Each test solution was applied in a volume of 1 mL. The details of the method used have been fully reported previously.[12]

Observation of Middle Ear In Vivo. Healthy male chinchillas (410 to 500 g) were used. The animals were free of middle ear infection and hearing loss as determined by otomicroscopic observation, tympanometry, and auditory brain stem electric response (ABR). Nineteen chinchillas were intraperitoneally anesthetized with sodium pentobarbital solution (25 mg/kg), and 1 mL of 10^{-5} mol/L PAF, 10^{-5} mol/L PGE2, 10^{-5} mol/L PAF and PGE2 ("PAF & PGE2"), or the control solution (0.1 v/v% methanol-PBS) was directly injected into the tympanic cavity through the superior bulla with a 27-gauge syringe under anesthesia. Nine chinchillas were injected with PAF into the bulla of the ear on one side, and also with PAF & PGE2 into the contralateral ear. Another two groups of 5 chinchillas were unilaterally injected with PGE2 and the control solution, respectively. At the time of each injection, we used an air-venting needle (27-gauge), which was inserted into the superior bulla to avoid perforating the tympanic membrane. The middle ear was examined by otomicroscopy, tympanometry, and ABR in relation to time. Tympanometry testing was performed immediately after injection, and on the 1st, 4th, 7th, and 10th days postinjection, and also, if required, on the 14th day. The ABR testing was performed on the 4th, 7th, and 10th days postinjection with a signal processor (SANEI 7S11A, San-ei, Tokyo, Japan). For acoustic stimulation, 4-kHz clicks of 90-microsecond duration were given through an earphone with a uniform stimulus interval of 75 milliseconds. A total of 128 clicks constituted a single average, and repeated recordings were made to check reproducibility.

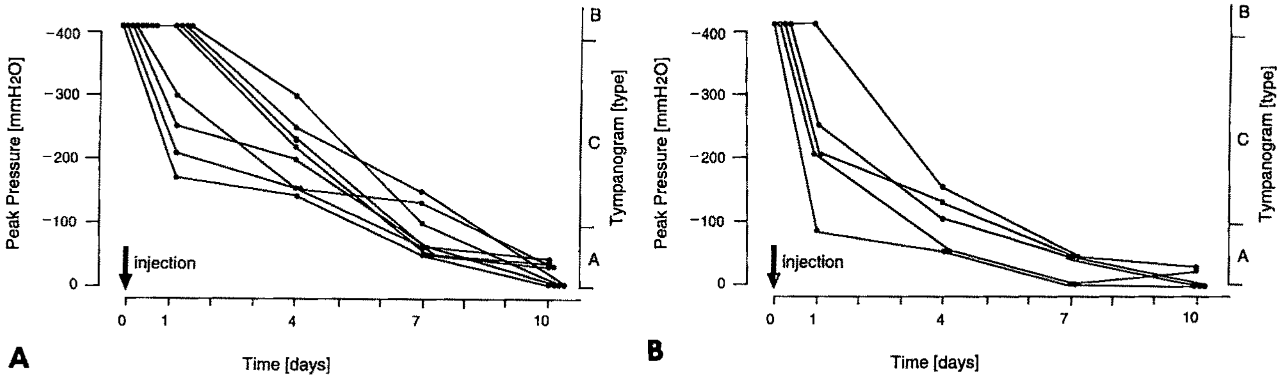
Chinchillas were used in these experiments because their large middle ears, which are surrounded by thin bone, facilitate examination.

Statistical Analysis. The significance of differences between recorded values was determined statistically by Student's *t*-test. A level of $p < .05$ was accepted as statistically significant.

RESULTS

Effect of PAF on Ciliary Activity In Vitro. The effects of PAF on ciliary activity of tubal mucosa are illustrated in Fig 1. The PAF induced ciliary inhibition at concentrations ranging from 10^{-10} to 10^{-6} mol/L in a dose-dependent manner. Ciliary activity was significantly inhibited by both 10^{-6} and 10^{-8} mol/L PAF after 2 hours of exposure, and by 10^{-10} mol/L PAF after 4 hours of exposure. However, the control solution (0.01 v/v% methanol–RPMI 1640) had no effect on ciliary activity. Observation of the effects of 10^{-6} and 10^{-8} mol/L PAF on ciliary activity was discontinued after 5 hours and 10 hours of exposure, respectively, because the number of exfoliated and/or ciliostatic cells reached more than 25% of all observed cells at these times.

Clearance of Middle Ear Effusion In Vivo. Before



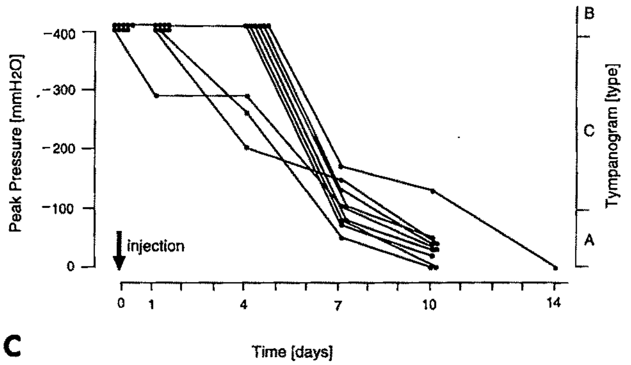


Fig 2. Time courses of tympanogram peak pressures in chinchillas. A) Injected with 1 mL of 10^{-5} mol/L PAF into tympanic cavities (n = 8). B) Injected with control solution (n = 5). C) Injected with 10^{-5} mol/L PAF & PGE2 (n = 9).

injection of test solution into the tympanic bulla, all 19 chinchillas (28 ears) exhibited normal tympanic membranes and type A tympanograms. The time courses of tympanogram peak pressures occurring when 10^{-5} mol/L PAF was injected into the tympanic cavities are illustrated in Fig 2A. All 8 tympanograms showed type B responses immediately after PAF exposure, and clear effusion was observed behind the tympanic membrane. We observed 8 of 9 ears injected with PAF. The remaining ear was omitted because of perforation of the tympanic membrane at the time of injection. On the 1st day postinjection, type B and type C responses were observed in 4 ears. Even on the 4th day, all tympanograms showed type C responses. The color of the tympanic membrane was amber in all ears, and serous foamy effusion was observed behind the tympanic membrane in 5 ears. On the 7th day postinjection, 5 of 8 ears were restored and showed type A responses. Another 3 ears showed a type C response, and the color of their tympanic membrane was slightly amber. It took 10 days for all ears to be completely restored and for tympanic membranes to appear normal.

The time courses of peak pressures of the control group are illustrated in Fig 2B. Even on the 1st day, 3 of 5 ears showed a type C and only 1 ear maintained a type B response. Furthermore, 1 ear had already been restored to type A by this time. On the 4th day, type A and type C responses were observed in 2 and in 3 ears, respectively. The color of the tympanic membrane was slightly amber in the type C group. All 5 ears had normal tympanic membranes and type A tympanograms within 7 days postinjection. The time courses of peak pressures of the PGE2 group were the same as those of the control (not shown), with 4 of 5 ears showing type A responses within 7 days.

The time courses of the group injected with both PAF and PGE2 are illustrated in Fig 2C. All tympanograms but one showed type B responses on the 1st day, and 6 of 9 ears maintained a type B response for 4 days. We observed serous foamy effusion behind the amber-colored tympanic membrane in all 9 ears. Five ears showed type C responses with slightly amber-colored tympanic membranes even on the 7th day, and all exhibited normal tympanic membranes and type A tympanograms within 14 days.

The mean and SE of each group is shown in Fig 3 in relation to time. A significant difference was observed between the PAF & PGE2 group and the control even on the 1st day postinjection. On the 4th day, there was a significant difference between the PAF group and the control, but not between the PGE2 group and the control. Moreover, a significant difference was observed between the PAF & PGE2 group and the PAF group. Even on the 7th day postinjection, both PAF & PGE2 and PAF groups were significantly different from the control group.

The threshold elevation of ABRs of each group in

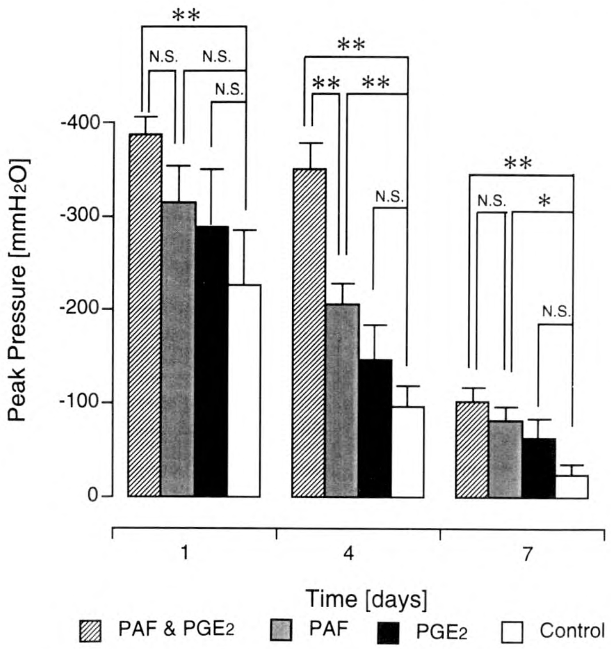


Fig 3. Tympanogram peak pressures at same time points in each group were compared. Values are expressed as mean ± SE. For PAF & PGE2, n = 9. For PAF, n = 8. For PGE2, n = 5. For control, n = 5. * — $p < .05$, ** — $p < .01$, N.S. — not significant.

relation to time is shown in Fig 4. The threshold average of ABRs of 16 chinchillas (22 ears) that we could examine was 19.3 ± 1.8 dB peak equivalent sound pressure level (peSPL) at preinjection. There was no significant difference between the threshold averages in the control, PGE2, PAF, and PAF & PGE2 groups. On the 4th day after injection, the averages of the threshold elevation of ABRs from baseline were 6.0 ± 4.2 dB in the control group and 9.0 ± 8.2 dB in the PGE2 group. No significant difference was observed between the control and PGE2 groups. In the PAF and PAF & PGE2 groups, the averages of the threshold elevation on the 4th day after injection were 24.2 ± 8.0 dB and 30.0 ± 17.0 dB, respectively. Both averages were significantly elevated in comparison with that of the control. On the 7th day, the averages of the threshold elevation were 3.0 ± 2.7 dB peSPL in the control group, 1.0 ± 2.2 dB in the PGE2 group, 5.8 ± 5.9 dB in the PAF group, and 9.0 ± 4.2 dB in the PAF & PGE2 group. Even on the 7th day, the threshold of the PAF & PGE2 group was significantly elevated compared with that of the control, but was restored on the 10th day postinjection.

DISCUSSION

Platelet activating factor inhibited ciliary activity of the eustachian tube mucosa of guinea pigs in vitro. The ciliary dysfunction was irreversible because exfoliation and/or ciliostasis of ciliated cells was observed after PAF exposure. The cytotoxic mechanism of PAF is dependent on increased membrane permeability of ciliated cells and edema of organelles.[12] Furthermore, these effects of PAF were blocked by specific PAF antagonists.[21,22] Moreover, in the in vivo study, there were significant differences between the PAF group and the control in both the tympanogram peak pressure and the threshold elevation of ABR. We concluded that PAF decreased ciliary beat frequency and changed the condition of the fluid layer, including volume and viscosity, which is induced through vascular permeability and hypersecretion by submucosal glands. Consequently, PAF inhibited mucociliary transport and caused inflammatory disorders in the tympanic cavity.

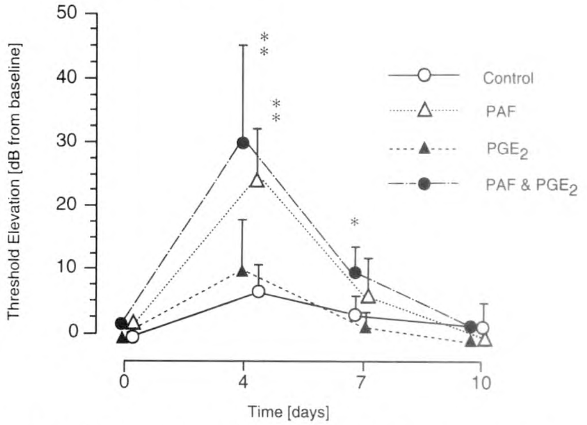


Fig 4. Time courses of auditory brain stem responses in chinchillas injected with 10^{-5} mol/L PAF & PGE2 (n = 6), 10^{-5} mol/L PAF (n = 6), 10^{-5} mol/L PGE2 (n = 5), or control solution (n = 5) into tympanic cavities. Values are expressed as mean ± SD. * — $p < .05$ versus control, ** — $p < .01$ versus control.

The mean peak pressure of the PGE2 group was lower than that of the control throughout the experimental period, but the difference was not significant. In our previous study, PGE2 promoted ciliary activity of the eustachian tube mucosa of guinea pigs in vitro,[15] and has been reported to activate ciliated cells by elevating their levels of cyclic adenosine 3′,5′-monophosphate (cAMP).[23] However, we would expect there to be no difference between PGE2 and the control in vivo, because PGE2 does not inhibit the mucociliary transport system. Not only does PGE2 induce vasodilatation, it also increases vascular permeability,[24] and these effects do not necessarily reduce the effusion in the tympanic cavity, despite the acceleration of ciliary activity.

In the present study, we observed that PGE2 augmented the inhibitory effect of PAF on mucociliary clearance. The PAF & PGE2 group always showed a lower mean tympanogram peak pressure than the PAF-only group, and the difference was significant on the 4th day after injection (Fig 3). The threshold elevation of ABR in the PAF & PGE2 group was also significantly different from that in the control group even on the 7th day; however, there was a significant difference between the PAF group and the control only on the 4th day (Fig 4). Prostaglandin E2 alone did not significantly inhibit mucociliary clearance from the middle ear compared with the control. However, vascular dilatation, which is the main biologic effect of PGE2, was suspected to have augmented the observed increase in vascular permeability and hypersecretion by submucosal glands induced by PAF. We observed synergistic effects of PAF and PGE2 in both tympanograms and ABRs.

Disorders in the middle ear induced by injection of PAF were observed by otomicroscopy, tympanometry, and ABR to show complete amelioration within 10 days postinjection. The effusion induced by PAF was more quickly transported and cleared from the tympanic cavity than that in experimental OME induced by endotoxin[25] or keyhole limpet hemocyanin.[26] Platelet activating factor is biologically unstable; its concentration was halved within 11.4 minutes and reduced almost to zero within 60 minutes in vitro.[22] A single injection of PAF did not lead to effusion remaining in the tympanic cavity over a long period.

The clearance of effusion from the middle ear is dependent on both the static function of mucociliary transport and the dynamic function of swallowing. It has been reported that patients with OME suffer from tubal dysfunction of mucociliary transport and ventilation.[27] Particularly, impaired ventilation function prevents the cancellation of negative pressure in the tympanic cavity. The ventilation function of chinchillas used in the present study was regarded as normal, because all tympanograms were type A prior to injection. The observed difference in clearance of effusion between the PAF group and the control in the present study was mainly dependent on mucociliary dysfunction induced by ciliary inhibition, glandular hypersecretion, and mucosal damage. Thus, PAF caused inflammatory disorders in the middle ear. Mucosal inflammation of the middle ear was observed in histopathologic studies of temporal bone when PAF was injected into the bullae of chinchillas.[28]

In the present study, we found that PAF inhibited mucociliary clearance of the eustachian tube from the middle ear. Furthermore, PGE2, a mediator that is always found in inflammatory disorders, augmented the inhibitory effects of PAF on mucociliary clearance. These findings indicate that PAF and PGE2 play important roles in accumulation of effusion in the middle ear.

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LARYNGEAL AND TRACHEAL RECONSTRUCTION

The Second International Symposium on Laryngeal and Tracheal Reconstruction will be held in Monte Carlo, Monaco, May 22-26, 1996. For more information, contact Tihana Barisic, Publi Creations, 44, Boulevard d'Italie, Monte-Carlo, MC, 98000 Monaco; telephone +33-93-159976; fax +33-93-159977.

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The international workshop Middle Ear Mechanics in Research and Otosurgery will be held in Dresden, Germany, September 19-22, 1996. For further information, contact Prof Dr K. B. Hüttenbrink, Universitäts-HNO-Klinik, Fetscherstr 74, 01307 Dresden, Germany; telephone (Germany) (0)351-458-2224; fax (0)351-458-4326.

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LACK OF EFFECT OF HOT, HUMID AIR ON RESPONSE TO NASAL CHALLENGE WITH HISTAMINE

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We have previously shown that whole body exposure of human subjects to environmental conditions of 37°C and 90% relative humidity (RH) prior to and during nasal challenge with antigen decreases the early response. In this study, we evaluated 1) whether the decreased responses observed resulted from decreased end organ sensitivity to histamine and 2) whether the effect of hot, humid air persisted after the subject exited the hot, humid environment. Ten asymptomatic seasonal allergic subjects and 11 nonallergic subjects were randomized to environmental chamber conditions of either 20°C, 30% RH or 37°C, 90% RH for 1 hour prior to and during performance of a nasal challenge with histamine. Twenty-two hours after exiting the environmental chamber, the allergic subjects were challenged with antigen. During both chamber conditions, histamine challenge was associated with a significant increase in all measured parameters compared to sham challenge, except for the sensations of pruritis and congestion. The response to histamine challenge was not different under the two experimental conditions or between allergic and nonallergic subjects. Following both exposure conditions, allergen challenge was associated with an increase in all measured parameters compared to sham challenge, with no significant difference between the two conditions. Exposure to 37°C, 90% RH minimally affects the response to nasal challenge with histamine, and thus, the previously reported decreases in the early nasal response to antigen may primarily result from reduction in mast cell activation. The effect on antigen does not persist 22 hours after exposure.

KEY WORDS — allergic rhinitis, anaphylaxis, heat, histamine, nasal provocation, nasal reflex, nasal secretions, perennial rhinitis.

INTRODUCTION

In normal subjects, inhaling hot air (39°C) decreases nasal airway resistance and tends to cause a decrease in mucociliary transport.[1] Olsson and Bende[2] showed in normals that 40°C air increased nasal patency without changing mucosal blood flow as assessed by the xenon washout technique.

Treatment of the nasal mucosa with supersaturated, 44°C air has been reported to have a beneficial effect on perennial allergic and nonallergic rhinitis. Three 30-minute treatments reduced symptoms and improved peak nasal expiratory flow in perennial rhinitics,[3-6] an effect lasting for 1 month.[3] Other investigators have not reproduced these findings.[7] Similarly, treatment with supersaturated, 44°C air has been reported to improve symptoms in naturally occurring and experimentally induced rhinovirus infections.[8] Other studies, both in experimental settings and in field settings, do not confirm this effect.[9-11]

In nasal antigen provocation studies performed in a laboratory setting, Johnston et al[12] showed that treatment with supersaturated water at 43°C prior to provocation reduced allergen-induced vascular permeability and nasal airway resistance (NAR). The same group of investigators also showed that local hyperthermia reduced exercise-induced asthma 1 hour, but not 24 hours, after exposure. Histamine challenge was not affected in this study.[13] In previous studies, we showed that whole body exposure of human subjects to 37°C, 90% relative humidity (RH) conditions prior to and during nasal challenge with antigen decreased biochemical, physiological, and subjective responses to challenge[14] (also Desrosiers et al, unpublished observations). Decreased levels of histamine after the challenge implied decreased mast cell degranulation. Neural activation was also decreased, as shown by decreases in the number of sneezes and the sensation of pruritis. These changes were accompanied by decreases in the subjective reporting of nasal congestion, as well as by a decrease in NAR and a decrease in the level of albumin, a marker of vascular permeability, recovered in nasal lavage. Secretory responses, as measured by secretion weights, lactoferrin (a marker of serous glandular secretion), and the subjective reporting of rhinorrhea, remained unchanged.

We wanted to determine whether the decreases in responses were secondary to decreased mast cell histamine release or whether air at 37°C, 90% RH (hot, humid air [HHA]) also altered sensitivity of the nasal end organs to histamine. A secondary goal of our study was to determine whether the protective

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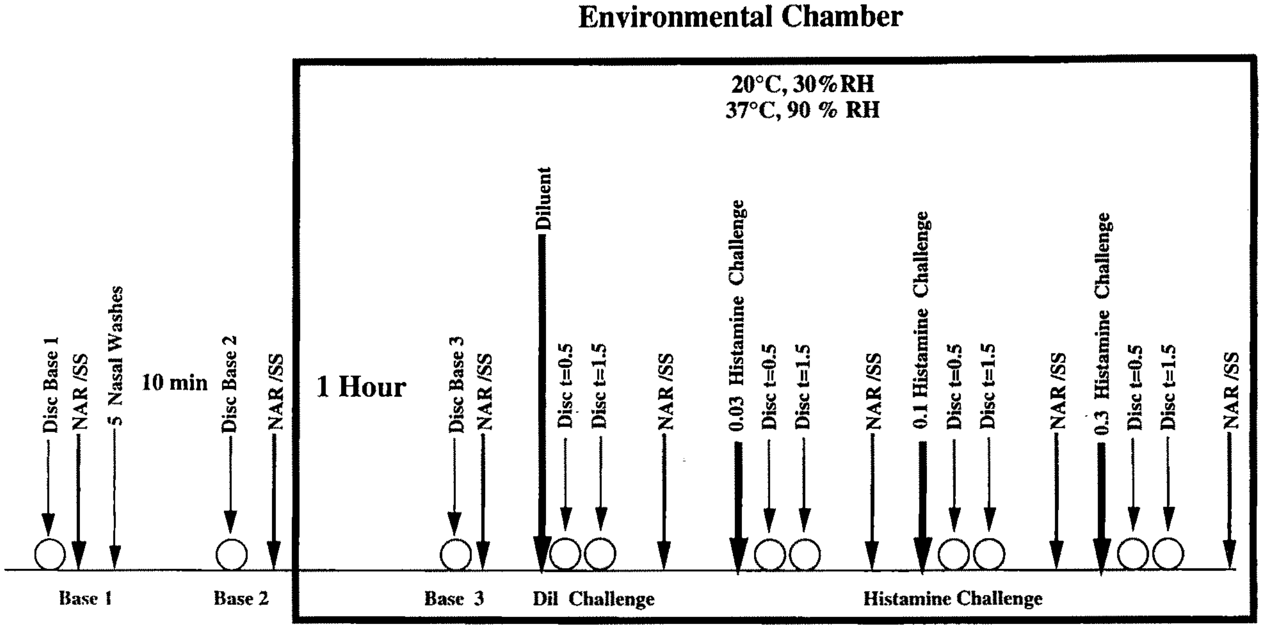


Fig 1. Experimental design. Time points for baseline (Base) measurements, nasal washes, entry into chamber, and collection of secretions; nasal airway resistance (NAR); and symptom scores (SS) after diluent (Dil) and histamine challenges are all indicated. RH — relative humidity; t = 0.5 — 30-second time point postchallenge; t = 1.5 — 90-second time point postchallenge.

effect of HHA against antigen persisted after the subject exited the environmental chamber.

MATERIALS AND METHODS

SUBJECTS

Nine seasonal allergic and 11 nonallergic volunteers were recruited (allergic: 4 men, 5 women, ages 28 to 40 years, mean 31 years; nonallergic: 5 men, 6 women, ages 24 to 34 years, mean 28 years). Allergic status was confirmed by positive skin puncture testing (≥3 mm wheal). Subjects allergic to *Dermatophagoides farinae* and *Dermatophagoides petronyssius* were excluded, as were those presenting nasal symptoms outside of their pollen season. All allergic subjects had previously reacted to nasal challenge with a relevant allergen by sneezing and increased nasal secretions, compared to a sham challenge with the diluent (phenol-buffered solution 0.5%, Greer Laboratories, Lenoir, NC) for the allergen challenge solution. Nonallergic subjects had negative skin puncture results on tests of a panel of common allergens. All subjects were free of symptoms at the time of study. No subject had had an upper respiratory tract infection within the preceding 2 weeks or had taken antihistamines within the past week. No subject had received astemizole within the past 3 months. Nasal steroids, cromolyn, and oral corticosteroids had not been received for at least 1 month. No significant septal deviation or other mechanical obstruction was present. Written informed consent was obtained from each subject prior to entry, and approval for this study was granted by the Institutional Review Board of the Francis Scott Key Medical Center.

EXPERIMENTAL PROTOCOL

We performed a prospective, randomized, crossover study comparing the effects of HHA and 20°C, 30% RH air (room air [RA]) on the response of the nasal mucosa to histamine provocation performed in an environmental chamber followed 22 hours later, in the allergic subjects, by a nasal challenge with antigen. At least 2 weeks separated the exposures. Subjects were randomized so that one half of the group underwent an HHA-RA series, and the other half an RA-HHA series. Because of the particular nature of the stimulus used, participants could not be blinded as to the nature of the environmental chamber; however, conditions were otherwise duplicated. An environmental chamber, large enough to accommodate the subject and the researcher (Environmental Sciences, Raleigh, NC) and capable of precisely reproducing temperature and humidity conditions, was used to create the ambient conditions. We chose the condition of RA because it resembles air typically found in climate-controlled environments, whereas air at 37°C, 90% RH (HHA) is similar to that found in the alveoli.

The protocol for the challenge is represented in Fig 1. First, filter paper discs were placed to collect nasal secretions, NAR was measured, and symptom scores were recorded by the subjects to establish a baseline (base 1). Five nasal washes with 10 mL of 0.9% saline (McGaw Inc, Irvine, Calif) were then performed to remove preexisting mediators. After 10 minutes, a second series of baseline measurements were made (base 2). Subjects then entered the environmental chamber, where they were seated for the remainder of the exposure. After 1 hour, a third series of baseline measurements were performed (base 3). Nasal challenge with diluent solution (0.9% saline [McGaw Inc]) was then performed to assess nonspecific hyperreactivity. The challenge was delivered by placing 50 µL of diluent on a filter paper disc and then applying the disc to the anterior nasal septum for 60 seconds under direct visualization by means of a duck-bill forceps. Thirty seconds after its removal, dry discs were applied bilaterally on the anterior nasal septum and left in place to collect nasal secretions for 30 seconds (time point $t = 0.5$ minute). By this method, secretions were collected at $t = 0.5$ and 1.5 minutes. After a 5-minute waiting period, NAR was recorded and symptom scores were recorded again. Histamine challenges employing the same technique followed, using 50 µL of 0.7 mg/mL, 2.2 mg/mL, and 7 mg/mL concentrations of histamine diphosphate (Sigma Chemical Inc, St Louis, Mo) for delivered quantities of 0.03 mg, 0.1 mg, and 0.3 mg of histamine, respectively. Secretions were then collected at the same time points as for diluent challenge. Choice of time points was based on previous experiments showing a rapid rise in secretion weights followed by a slower rise in albumin. Albumin was assayed from secretions collected at the third baseline and from time points $t = 0.5$ and $t = 1.5$ minutes after both the diluent and 0.3 mg histamine challenges. The subjects remained in the chamber for approximately 1 hour 45 minutes.

To evaluate if protective effects shown in the prior study persisted following the exposure period, we performed an allergen challenge in the allergic population 22 hours after they exited the chamber. The antigen challenge protocol began with a sham challenge using 50 µL of the vehicle for the allergen solution (0.5% phenol-preserved saline, Greer Laboratories). Secretions were collected at 0.5, 1.5, and 2.5 minutes. Nasal airway resistance was measured and symptom scores were recorded. Allergen challenge employing the same technique as for the histamine challenge followed, using 50 µL of either ragweed or mixed grass (timothy, June, meadow fescue, and orchard) extract at a concentration of 6,667 protein nitrogen units per milliliter (Greer Laboratories) for a delivered quantity of 333 protein nitrogen units (approximately 1.2 µg of Amb a 1 for ragweed solutions).

COLLECTION OF NASAL SECRETIONS

Eight-millimeter discs punched from filter paper (No. 190005, Shandon, Pittsburgh, Pa) were used both for collection of secretions and for delivery of the challenge solutions, as previously described.[15] Briefly, 8-mm discs were punched from filter paper and placed in 1.5 mL Eppendorf tubes (Sarstedt, Newton, NC). These disc-tube combinations were weighed (Mettler analytical balance AE240, Mettler Instruments, Highstown, NJ). The discs were removed immediately prior to being placed on the nasal septum for a period of 30 seconds to collect secretions. Following removal, they were immediately replaced in the Eppendorf tube. The disc-tube combination was again weighed, and the postcollection weight was subtracted from the precollection weight to determine the amount of secretions collected in the 30-second time period. Biochemical measurements were also done in a blinded fashion.

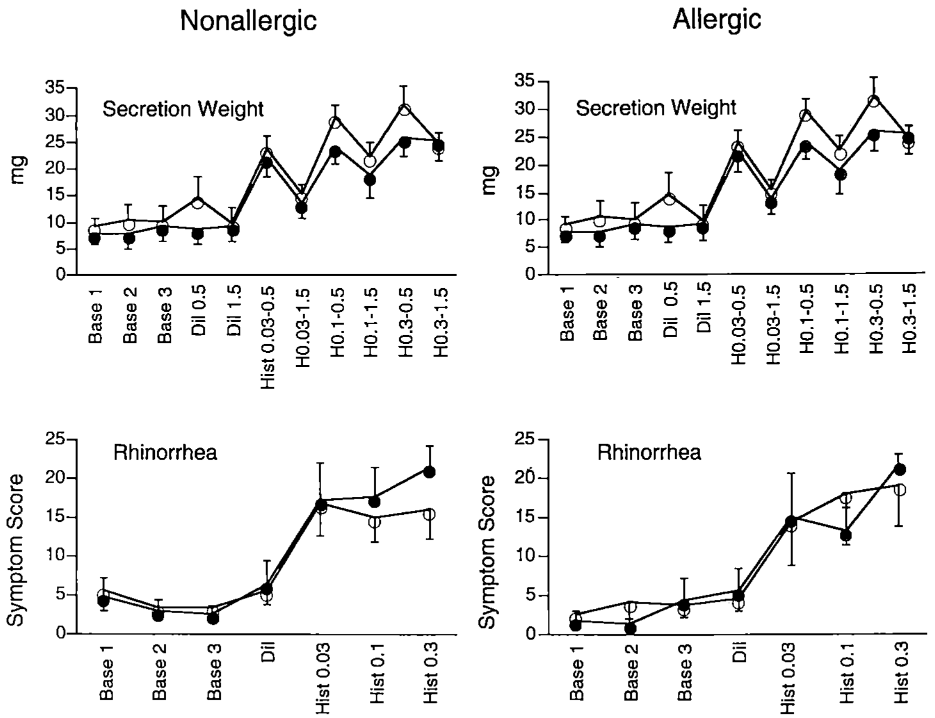
ALBUMIN ASSAY

Human serum albumin is measured with a noncompetitive enzyme-linked immunosorbent assay. Albumin was eluted off the collection discs by incubation in 300 µL of phosphate-buffered saline at room temperature for 30 minutes. Following elution, eluates were stored at −20°C until assayed. All samples from an individual were run in the same assay to avoid interassay variability. For each assay, a 1:900 dilution of a sheep antibody to human serum albumin (The Binding Site, Inc, San Diego, Calif) was immobilized on microtiter well plates overnight. Plates were washed and nonspecific binding sites blocked by incubation with 1% sheep serum for 30 minutes at room temperature. After washing the plate, standard albumin or samples at various dilutions were added to the plate and incubated for 90 minutes at 37°C. After washing, the plates were incubated for a further 90 minutes at 37°C with a 1:1,000 dilution of a second sheep anti human albumin antibody conjugated to horseradish peroxidase (Dako Corp, Carpinteria, Calif). After washing, the reaction was developed with 1 mmol/L 2,2′azino-di-[3 ethylbenthiazone sulfonic acid] as a substrate, and absorbance was read at 410 nm on a plate reader. The assay standard curve ranges between 1 and 256 ng/mL. This assay has been shown to provide excellent agreement with a published radioimmunoassay[16] that was previously used to assay albumin in nasal lavages. Albumin levels are reported as total quantity recovered (the albumin concentration in the eluate multiplied by the volume of secretions plus eluate). One microgram of secretions was assumed to be equivalent to 1 µL for this calculation.

NASAL AIRWAY RESISTANCE

Nasal airway resistance was measured at rest over 10 seconds of closed-mouth breathing by anterior rhinomanometry (Allergopharm A440 digital anterior rhinomanometer, Allergopharma Joachim Ganzer, Hamburg, GmbH, Hamburg, Germany). Results

Fig 2. Secretory responses. Shows time course of secretion weights and rhinorrhea on days in room air (●) and in hot, humid air (○). Results for nonallergic subjects (n = 11) are shown on right and allergic subjects (n = 9) on left. Time is plotted on x-axis and data are shown for baselines (Base) and for diluent (Dil) and allergen challenges. Data are plotted as individual points to emphasize that multiple time points were collected after each challenge. There are no differences between two conditions for either group. Hist 0.03-0.5 — histamine challenge with 0.03 mg, 30-second time point; Hist 0.3-1.5 — histamine challenge with 0.03 mg, 90-second time point.



are reported in kilopascals per liter per second.

SYMPTOM SCORES AND SNEEZES

Symptoms were recorded by the subject on a visual analog scale. Rhinorrhea and congestion were evaluated separately for each nostril, and pruritis was evaluated in a combined fashion for the nose and throat. Subjects made a vertical mark through a 100-mm line to indicate their symptoms, ranging from no sensation at all to the worst possible sensation imaginable. Scores were obtained by measuring the distance along the scale in millimeters. Sneezes were counted by the investigator.

DATA ANALYSIS

Change in parameters following exposure to the environmental condition were obtained by comparing baseline measurements after the nasal washes (base 2) to the values measured after exposure to HHA or RA prior to diluent challenge (base 3). For

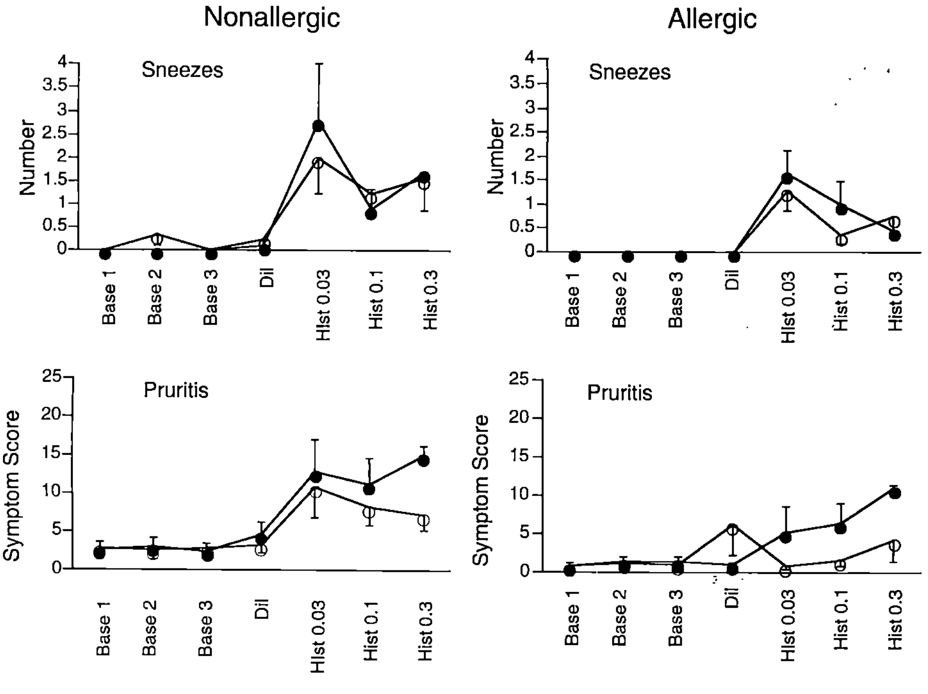


Fig 3. Neural responses. Sneezes and pruritis on room air (●) and hot, humid air (○) days are plotted. There are no significant differences between two conditions in either group. For abbreviations, see Fig 2.

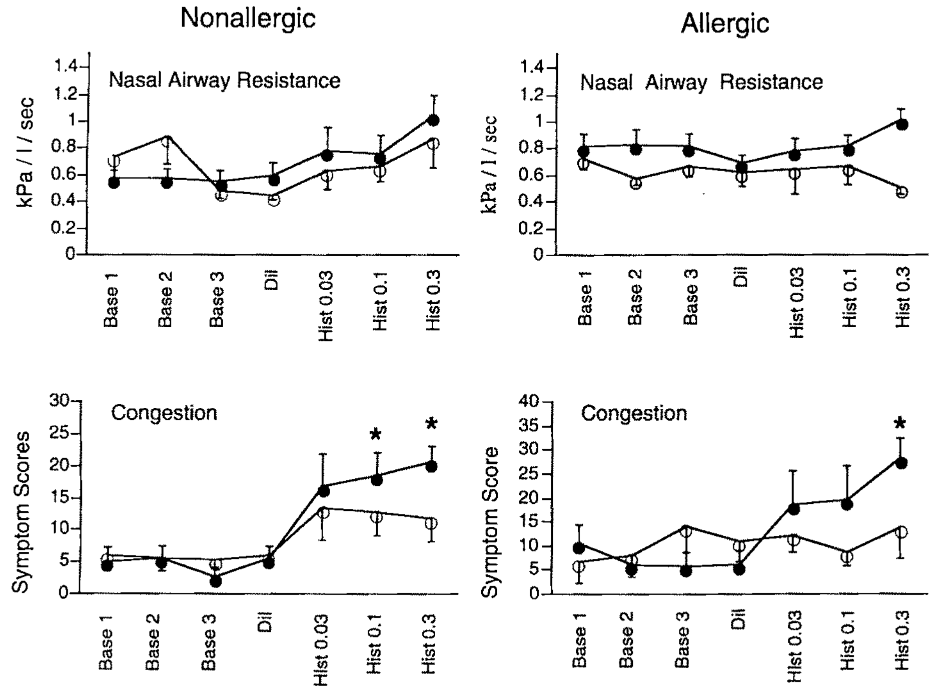


Fig 4. Congestive responses. Shows time course of nasal airway resistance, albumin (for allergics only), and congestion on room air (●) and hot, humid air (○) days. Only significant differences are in sensation of congestion for 0.1- and 0.3-mg histamine challenge of allergic subjects and for 0.3-mg histamine challenge for nonallergic subjects. * — $p < .05$. For abbreviations, see Fig 2.

both diluent and each of the histamine challenges, secretion weights and albumin are expressed as the sum of the values for each of the time points after challenge. Nonspecific responses to sham challenge were obtained by subtracting values at base 3 from those induced by challenge with the diluent solution. Changes in parameters to histamine challenges were obtained by subtracting the value following diluent challenge from that following each histamine challenge. Total change in response to histamine challenge was obtained by adding the results from the three histamine challenges. Allergen challenges were analyzed in a similar fashion. Total changes in response to allergen challenge were obtained by subtracting the response to diluent challenge from the response to allergen challenge. Statistical analysis was performed with nonparametric methods. For descriptive purposes, data are reported as means ± SEM. Friedman's ANOVA was applied to each series of measurements following histamine challenge. Changes from time points base 2 to base 3, base 3 to diluent, and diluent to histamine challenges were evaluated with a Wilcoxon signed rank analysis, as were differences between individual points and totals for the HHA and RA conditions. Allergic and nonallergic populations were compared by means of a Mann-Whitney *U* test. Significance was defined as $p \le .05$ with two-tailed p values. All statistical analyses were performed with the Statview II data analysis program (Abacus Concepts Inc, Berkeley, Calif) on a Macintosh IIci microcomputer equipped with a math coprocessor (Apple Computer, Cupertino, Calif).

RESULTS

HISTAMINE CHALLENGES

Allergic Subjects. Baseline levels of all measured parameters were comparable on both days. These remained unaffected by the nasal lavages and by the initial conditioning period. No significant increases over baseline were recorded following diluent challenge. Histamine challenge provoked marked increases over diluent for all ipsilateral parameters in both RA and HHA. Secretory response increased, as shown by increases in total change in secretion weight (RA: 17.4 ± 4.80 mg [sum of time points collected] for diluent to 51.1 ± 6.8 mg for 0.3-mg histamine challenge, $p \le .01$; HHA: 24.5 ± 6.0 mg to 56.5 ± 3.2 mg, $p \le .01$) and rhinorrhea (RA: 6.3 ± 3.1 score to 21.5 ± 5.2 score, $p \le .01$; HHA: 5.6 ± 1.9 score to 16.1 ± 3.8 score, $p \le .01$; Fig 2).

Sneezing increased (RA: 0.1 ± 0.1 sneezes versus 1.7 ± 0.6 sneezes, $p \le .01$; HHA: 0.2 ± 0.1 sneezes versus 1.6 ± 0.7 sneezes, $p \le .01$), as did the sensation of pruritis (RA: 4.5 ± 1.7 score to 15.0 ± 4.7 score, $p \le .01$; HHA: 3.1 ± 1.0 score to 7.2 ± 2.0 score, $p \le .01$; Fig 3).

The NAR increased (RA: 0.6 ± 0.1 kPa/L per second to 1.1 ± 0.3 kPa/L per second, $p \le .01$; HHA: 0.5 ± 0.04 kPa/L per second to 0.87 ± 0.21 kPa/L per second, $p \le .01$), and was accompanied by increases in albumin (RA: 47.6 ± 17.0 mg to 194.4 ± 20.7 mg, $p \le .01$; HHA: 31.4 ± 8.7 mg to 198.8 ± 28.8 mg, $p \le .01$) and congestion (RA: 5.5 ± 2.2 score to 20.6 ± 4.5, $p \le .01$; HHA: 6.0 ± 2.0 score to 11.8 ± 4.0, $p \le .01$; Figs 4 and 5).

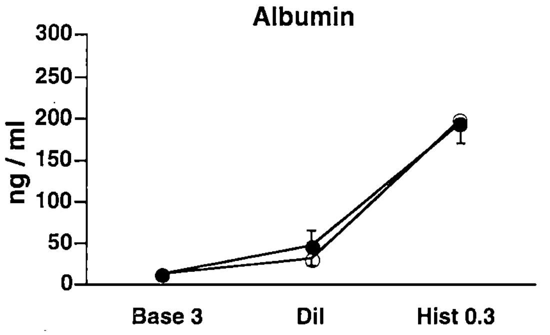


Fig 5. Time course of albumin concentration for allergic subjects only on room air (●) and hot, humid air (○) days. There are no differences between two conditions. For abbreviations, see Fig 2.

There was no difference in the responses to histamine challenge between RA and HHA days, with the exception of congestion, which was less in HHA (p ≤ .05; Table 1).

Normal Subjects. The response of normal subjects differed slightly from that of allergic subjects. Baseline levels were low and remained unchanged by nasal lavage. Conditioning with RA or HHA had no effect, nor did diluent challenge. As with allergic subjects, histamine challenge produced an increase over diluent for all the parameters on both RA and HHA days, except for the sensations of pruritis and congestion, which did not increase when histamine challenge was performed in HHA.

Secretion weights increased (RA: 8.2 ± 1.0 mg for diluent to 31.7 ± 5.5 mg for 0.3-mg histamine challenge, p ≤ .01; HHA: 11.4 ± 2.5 mg to 37.0 ± 3.7 mg, p ≤ .01), as did rhinorrhea (RA: 11.4 ± 6.1 score to 21.7 ± 5.15 score, p ≤ .01; HHA: 4.6 ± 1.6 score to 19.0 ± 5.3 score, p ≤ .01; Fig 2).

Sneezing increased (RA: 0.0 ± 0.0 sneezes versus 0.5 ± 0.2 sneezes, p ≤ .01; HHA: 0.0 ± 0.0 sneezes versus 0.7 ± 0.4 sneezes, p ≤ .01). In contrast to allergic subjects, there was no increase in the sensation of pruritis in normal subjects when histamine challenge was performed in HHA (RA: 0.9 ± 0.3 score to 11.1 ± 5.2 score, p ≤ .01; HHA: 6.3 ± 4.2 score to 4.1 ± 2.7 score, p ≤ .05; Fig 3).

There was an increase in NAR following histamine challenge (RA: 0.69 ± 0.06 kPa/L per second to 1.01 ± 0.17 kPa/L per second, p ≤ .01; HHA: 0.62 ± 0.10 kPa/L per second to 2.06 ± 1.04 kPa/L per second, p ≤ .01) that was not accompanied by an increase in congestion (RA: 6.0 ± 2.8 score to 28.2 ± 6.9, p ≤ .01; HHA: 10.7 ± 4.2 score to 13.8 ± 6.3, p ≤ .28; Fig 4).

Comparing responses of normal subjects to histamine challenges between RA and HHA days, a pattern similar to that of allergics was found (Table 1).

TABLE 1. HISTAMINE CHALLENGE

	Allergics			*Normals*		
	RA	*HHA*	*p*	*RA*	*HHA*	*p*
Secretion weights						
Mean	77.52	73.97	.86	61.11	55.34	1.00
SEM	6.22	20.59		9.29	10.97	
Rhinorrhea						
Mean	37.5	31.33	.34	33.91	27.91	1.00
SEM	7.56	4.80		11.67	10.33	
Sneezes						
Mean	5.10	4.11	.91	3.09	2.36	.23
SEM	1.69	1.38		0.89	0.78	
Pruritis						
Mean	25.60	16.89	.25	19.82	–11.09	.12
SEM	9.54	6.34		10.71	12.77	
Albumin						
Mean	146.73	167.34	.96	ND	ND	
SEM	22.19	26.85				
Nasal airway resistance						
Mean	1.60	0.81	.55	0.57	3.00	.13
SEM	0.99	0.30		0.20	2.16	
Congestion						
Mean	39.5	19.78	.04	44.09	2.36	.05
SEM	11.03	6.24		15.85	10.84	

Shows sum of total change from diluent challenge for histamine challenge.

RA — room air (20°C, 30% relative humidity), HHA — hot, humid air (37°C, 90% relative humidity), ND — not done.

TABLE 2. ALLERGEN CHALLENGE

	RA	*HHA*	*p*
Secretion weights			
Mean	55.43	47.33	.86
SEM	10.02	10.96	
Rhinorrhea			
Mean	16.67	15.89	.95
SEM	4.02	3.28	
Sneezes			
Mean	2.56	3.44	.23
SEM	0.91	0.59	
Pruritis			
Mean	7.67	9.11	.48
SEM	3.48	2.08	
Nasal airway resistance			
Mean	1.89	1.11	.68
SEM	0.97	0.61	
Congestion			
Mean	11.56	19.00	.06
SEM	2.98	3.91	

Shows total changes from diluent challenge for ipsilateral allergen response.

RA — room air (20°C, 30% relative humidity), HHA — hot, humid air (37°C, 90% relative humidity).

There was less congestion when histamine challenge was performed in HHA. Secretion weights, rhinorrhea, sneezes, pruritis, albumin, and NAR were not affected by the environmental chamber conditions (Table 1).

Comparison of Normal and Allergic Subjects. Baseline measurements and responses to histamine challenge were compared between the allergic and normal populations (Table 1). Despite a trend toward worse nasal function in allergic subjects, baseline measurements were not statistically different between the groups. On both the RA and HHA days, responses to exposure in the chamber, diluent challenge, and allergen challenge were similar for both groups. The sole exception was the sensation of congestion in response to histamine challenge at the 0.3 mg concentration, which was less for allergic subjects in both HHA and RA conditions.

ALLERGEN CHALLENGE

The response to allergen challenge 22 hours after exiting the chamber was similar on both days (Table 2 and Fig 6). Baseline measurements were similar on both days and remained unchanged following lavage. Diluent challenge did not produce a significant change from baseline levels. Allergen challenge provoked significant increases over baseline in sneezing, symptom scores, secretory volume, and NAR. There was no difference between the conditions of HHA and RA with respect to the responses to allergen challenge

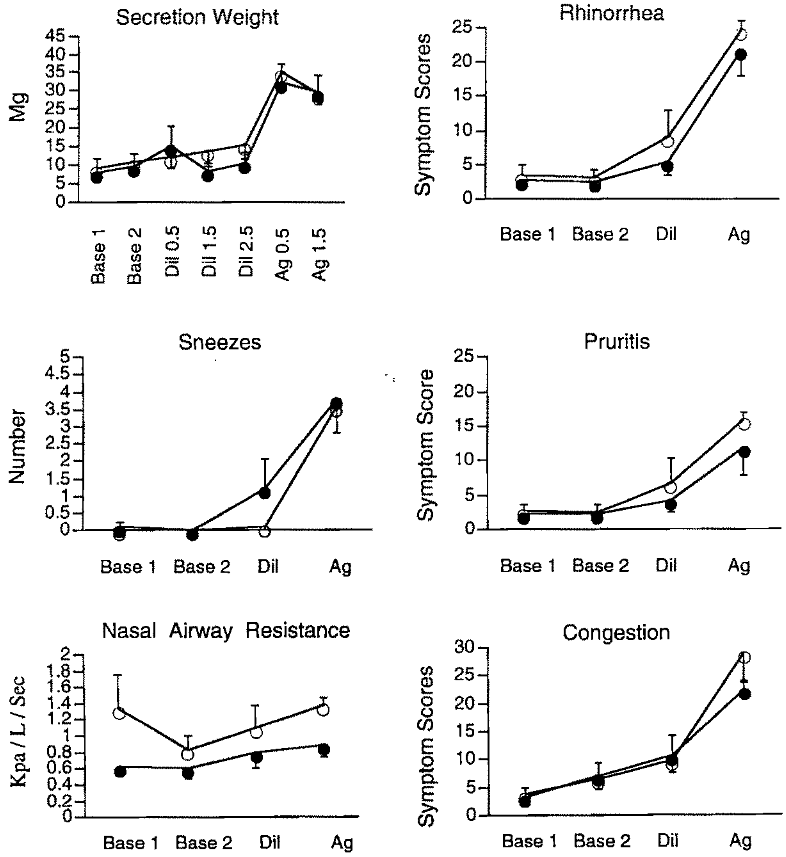


Fig 6. Allergen challenge (n = 9). Shows time course for different parameters following allergen challenge on allergic subjects on room air (●) and hot, humid air (○) days. Time is plotted on x-axis and data are shown for baselines and for sham and allergen challenges. Data are shown plotted as individual points in order to emphasize that multiple time points were collected after both antigen (Ag) and sham challenges. There are no differences between two conditions for either group. For other abbreviations, see Fig 2.

after 22 hours.

DISCUSSION

Previous studies showed that conditioning in an HHA environmental chamber decreased histamine release, as well as the physiologic response to nasal challenge[14] (also Desrosiers et al, unpublished observations). The results were consistent with decreased mast cell degranulation, with or without an alteration of sensitivity of the nasal end organs to histamine. This study addressed the contribution of the latter parameter. The results are clear. Conditioning the inspired air to 37°C, 90% RH did not affect the response to nasal challenge with histamine. This observation was true for both allergic and normal subjects. The sole exception was the ipsilateral sensation of congestion, found to be significantly reduced for both normal and allergic subjects. The decreased response to allergen challenge observed previously can thus be attributed to decreased mast cell activation, rather than to altered sensitivity to histamine. Whether the response to other mediators would be similar under different environmental conditions remains an open question.

Nasal congestion and NAR have been shown to be affected by changes both in whole body temperature and in the temperature of inspired air. Lundqvist et al[17] showed that increasing core body temperature by immersion in a hot water bath led to an increase in nasal patency as measured by acoustic rhinomanometry. Proctor et al,[1] however, showed that breathing air at 39°C did not increase core temperature. We did not monitor core temperature during this study. Olsson and Bende[2] did show that mucosal inhalation of 42°C air (RH not stated) increased nasal patency. Our baseline measurements of NAR were within normal limits and remained unchanged by exposure conditions. Our observation that HHA reduced the subjective reporting of congestion following histamine challenge may reflect this phenomenon. Since there were no objective changes as measured by anterior rhinomanometry, the strength of the subjective observation is decreased.

The mechanism by which HHA reduces mast cell activation has not been defined, but the most likely explanations involve changes in mucosal temperature or osmolality. There exists experimental evidence to support both possibilities. Temperatures of 40°C reduce both mast cell[18] and basophil histamine release.[19] Increased temperature has also been shown to reduce the production of cytokines,[20] induce the formation of heat shock proteins,[21] and potentiate the actions of interferons.[22] Hyperosmolar challenges produce stimulation of sensory nerves, blood vessels, and mast cells. Hot, humid air has been shown to lower the osmolality of tracheal secretions in dogs.[23] Thus, HHA could decrease osmolar load and effect a synergism with another stimulus. Regardless of the explanation, the different responses to antigen at RA and HHA suggest that the responsiveness of the lower airway to antigen provocation will be reduced relative to the upper airway because of the different airstream temperatures and relative humidities.

Johnston et al[12] compared allergen challenge performed 30 minutes after a 30-minute period of nasal breathing of supersaturated air at either 31°C or 42°C. They showed decreased vascular leakage and nasal airway resistance after pretreatment with the fully saturated, 42°C air, but did not find any effect on mast cell tryptase, sneezes, or mucus production. This result differs from our previous findings of a study in which we recorded decreases in histamine and sneezes following allergen challenge performed under HHA. We believe this is due to differences in the experimental design. We used a whole body exposure model and performed the challenge under HHA conditions, rather than 30 minutes postexposure. Our current observation regarding antigen provocation suggests that the effects of conditioning are transient, and thus, leaving the exposure condition for even 30 minutes may reduce its potency. We also used air with less water content.

There were no intergroup differences between the asymptomatic allergic and normal subjects, either at baseline or in response to histamine challenges. McLean et al[24] performed a series of nasal and bronchial challenges in atopic and nonatopic subjects with both histamine and methacholine, comparing NAR and forced expiratory volume in 1 second, respectively. Despite differences in bronchial sensitivity to both these agents, no intergroup differences could be found for nasal challenge. Other investigators have shown hyperresponsiveness of allergic subjects, but these studies used symptomatic rhinitic subjects rather than the asymptomatic subjects we studied. For example, White[25] found increased responsiveness to methacholine provocation in atopic individuals. She showed a decrease in the concentration of methacholine required to produce a 60% increase in the level of lactoferrin in nasal secretions, as recovered by nasal lavage.

Twenty-two hours after the subjects exited an HHA environment, its protective effect on nasal challenge with antigen was lost. This finding is in distinct contradiction with previously reported findings by Ophir et al,[5] who demonstrated a lasting effect on symptoms for up to 1 month. Other than by the conditions of exposure, we cannot explain the discrepancy. We are confident in our results, since the objective, subjective, and biochemical results were all in accord.

In sum, there appears to be no difference in the response to nasal challenge with histamine whether performed in RA or HHA or whether normal or allergic subjects are studied. We conclude that exposure to 37°C, 90% RH air decreases the early nasal response to antigen primarily by reducing mast cell activation, and not by altering sensitivity to histamine. This effect does not persist 22 hours after exposure.

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BIFID EPIGLOTTIS

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The true bifid epiglottis is a rare congenital anomaly typically discovered during the evaluation of stridor in an infant or newborn. While it is not classified as a specific syndrome, there are frequent associations of other congenital anomalies with the bifid epiglottis. These include midline defects (such as microphallus, hypospadius, imperforate anus, and midline laryngeal cleft), endocrine disorders (including congenital hypopituitarism), and central nervous system neoplasms, including hypothalamic hamartoblastoma. The embryogenesis and options for surgical management of this anomaly are reviewed, and one case is presented in detail.

KEY WORDS — bifid epiglottis, congenital anomalies, stridor.

The true bifid epiglottis is a rare congenital laryngeal anomaly that consists of two distinct cartilaginous halves.[1] More commonly, an epiglottis with a submucosal bifid appearance or a notching of the superior border is noted incidentally on direct laryngoscopy. Unlike these less complete forms, a true bifid epiglottis may present with upper airway obstruction. While the true bifid epiglottis can occur as an isolated airway anomaly, it can also occur in association with other midline anomalies, including congenital panhypopituitarism and hypothalamic hamartoblastomas.

CASE REPORT

A full-term male infant was born after an uneventful pregnancy. Apgar scores of 1 and 2 prompted immediate intubation and ventilation. The infant had multiple congenital anomalies, including midfacial hypoplasia, midline cleft lip and palate (see Figure, A), microphallus and cryptorchidism, and postaxial polydactyly (see Figure, B). Further genetic and endocrine evaluations revealed a normal chromosomal karyotype and congenital panhypopituitarism with secondary hypothyroidism and hypoadrenalism. Direct laryngoscopy and bronchoscopy revealed a true bifid epiglottis with an anterior midline cleft larynx (see Figure, C).

Over the subsequent 2 years, the patient required multiple intubations for respiratory distress. This ultimately resulted in subglottic stenosis that required placement of a tracheostomy tube. Because of persistent aspiration, a gastrostomy tube was placed. Severe gastroesophageal reflux disease necessitated Nissen fundoplication.

DISCUSSION

A notch or a small indentation of the superior border of the epiglottis and, to a lesser degree, an epiglottis with a submucosal bifid appearance is a frequent occurrence. The designation of a *true* bifid epiglottis is made when the anomalous epiglottis has two distinct cartilaginous halves with a complete midline cleft extending the entire length of the structure. A true bifid epiglottis rarely presents as an incidental finding on laryngoscopy, but rather is found during evaluation for stridor and/or aspiration. Multiple congenital anomalies have been associated with this finding, as the above case report highlights. The bifid epiglottis has also been associated with potentially life-threatening endocrine disorders.[2]

A review of the literature reveals only a few reports of this rare laryngeal anomaly. Authors detail the presenting symptoms and the associated congenital anomalies, but there is a paucity of information regarding the pathogenesis and treatment of the bifid epiglottis. In an early case report by Montreuil,[1] the history, pathologic findings, and subsequent clinical course were reported on a stridorous infant with a bifid epiglottis. Tracheostomy was the initial treatment, but the infant was decannulated after amputation of the entire epiglottis. In another reported case,[3] the presenting symptom was aspiration, not respiratory compromise. This patient had another midline congenital anomaly, an imperforate anus, as well as the absence of multiple digits. Management was conservative, with the spontaneous resolution of the symptoms over 3 years. Healy et al[4] reported two cases of bifid epiglottis associated with other congenital anomalies. In one, the bifid epiglottis was

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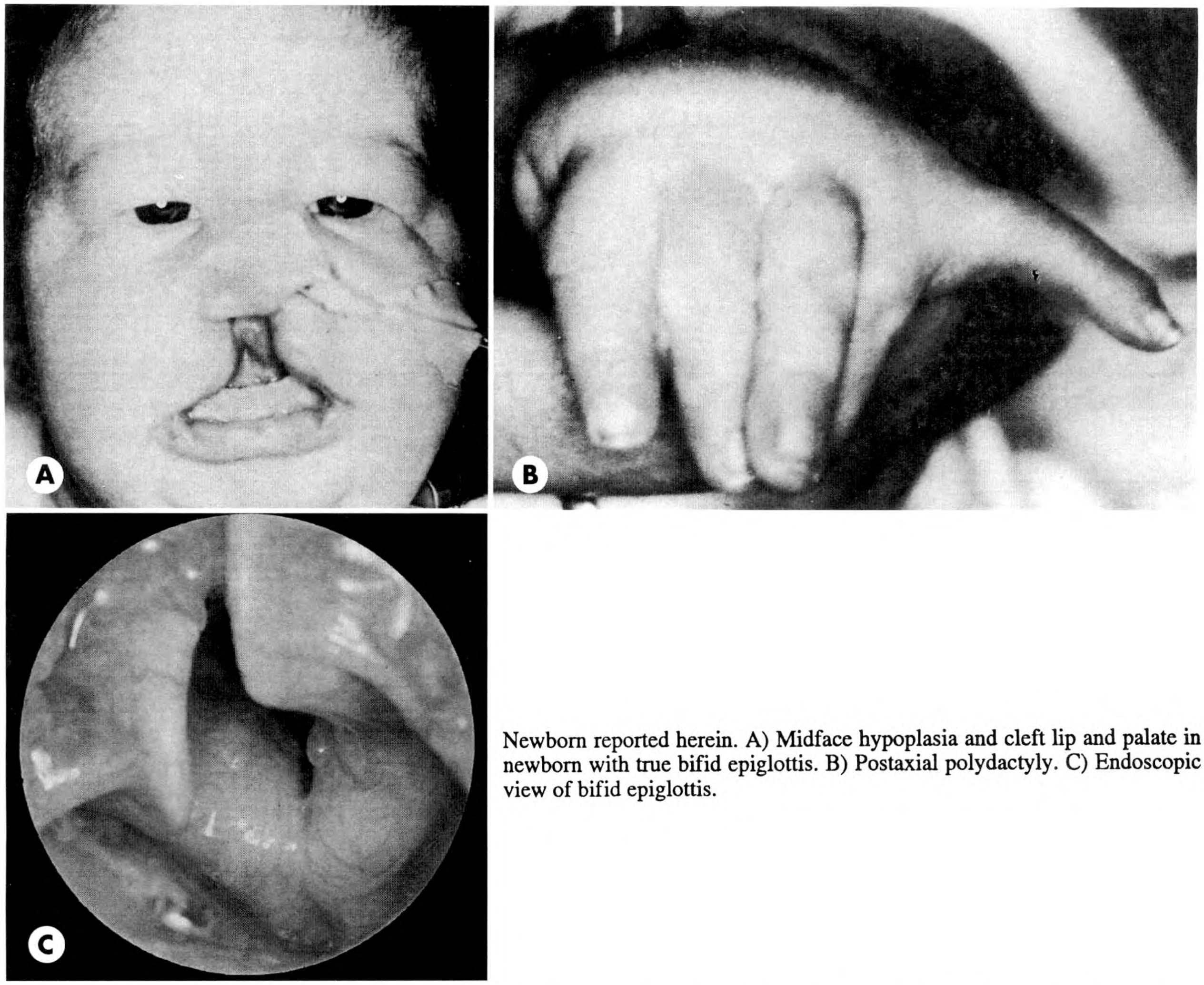

Newborn reported herein. A) Midface hypoplasia and cleft lip and palate in newborn with true bifid epiglottis. B) Postaxial polydactyly. C) Endoscopic view of bifid epiglottis.

found incidentally during an evaluation for acquired subglottic stenosis and was associated with polydactyly, imperforate anus, hypospadias, and cryptorchidism. A second case was associated with laryngeal cysts and polydactyly.

There have been a few reported attempts at surgical correction of a true bifid epiglottis. Montreuil[1] described amputation of the entire epiglottis as a means to eliminate airway compromise. Information relative to the development of chronic aspiration was not provided. A recent case report[5] describes management of a patient with bifid epiglottis utilizing microscopic laryngoscopy and carbon dioxide laser. During the initial evaluation for stridor, the patient had inspiratory collapse of the two distinct halves of the bifid epiglottis into the laryngeal inlet. Also noted was foreshortening of the aryepiglottic folds and prominent cuneiform tubercles, which apparently exacerbated the inspiratory collapse of the supraglottic structures. This patient was initially treated by endoscopic suturing of the two halves of the epiglottis to the tongue base in conjunction with carbon dioxide laser trimming of the lateral epiglottis, the aryepiglottic folds, and cuneiform cartilages. While the procedure was initially successful, inspiratory stridor returned after 3 months. Repeat endoscopy revealed epiglottic collapse on inspiration. Partial epiglottectomy was successful, according to the 2-year clinical evaluation. No mention was made of the feeding status or whether the patient was evaluated for aspiration.

Congenital hypopituitarism has been recognized to occur with clefts, polydactyly,[6] hypoglycemia, and genital hypoplasia. Early diagnosis and treatment can prevent the sequelae of short stature, hormonal imbalance, and metabolic instability.[7-9] A syndrome of panhypopituitarism in association with hamartomas of the central nervous system was described by Hall et al,[10] and is associated with a high rate of mortality. A bifid epiglottis appeared as a feature in a large percentage of these cases. Other features of the six patients described by Hall et al included imperforate anus, polydactyly, microphallus, midline clefts, and midfacial defects. A series by Graham et al[11] reported three cases of a true bifid epiglottis associated with congenital hypothalamic hamartoblastoma syndrome. These patients had a high mortality rate (66%) secondary to the unrecognized hypopituitarism. The third patient, having received a prompt diagnosis of panhypopituitary disorder, was treated and did well. New-onset seizures led to the eventual diagnosis of a hypothalamic hamartoblastoma.

The exact embryologic origin of the epiglottis remains controversial.[12-14] Commonly, the embryogenesis of the epiglottis is described as a fusion of the hypobranchial eminence and the ventral ends of the third and fourth arches. Since the hypobranchial eminence is a central structure, these mesodermal derivatives would fuse laterally. This makes an explanation of a true anterior midline cleft difficult, since a failure of fusion would not account for the resultant anomaly. A midline fusion of the third and fourth branchial arches would more logically result in this deformity, thus revising the previous embryologic conception of the hypobranchial eminence. Of interest, the epiglottis and the hypothalamus both develop between the 32nd and 42nd days of gestation.[10] This may account for the frequent association of hypothalamic hamartoblastomas, panhypopituitarism, and bifid epiglottis.

The true bifid epiglottis is an interesting laryngeal defect that appears within a range of clinical scenarios, ranging from an isolated airway anomaly causing respiratory embarrassment to one of a constellation of congenital anomalies associated with lethal central nervous system malformations. Our limited understanding of the cause of these anomalies makes it difficult to categorize these cases into one syndrome label.[15] However, the clinician caring for a patient with a true bifid epiglottis should be alert for the following associations.

1. A bifid epiglottis and midfacial cleft are often associated with other midline defects. These include microphallus, hypospadias, imperforate anus, and midline laryngeal cleft.

2. A bifid epiglottis and persistent hypoglycemia should prompt an endocrine evaluation for congenital panhypopituitarism.

3. Bifid epiglottis with postaxial polydactyly suggests the diagnosis of congenital panhypopituitarism.

4. The association of bifid epiglottis and congenital panhypopituitarism should prompt a computed tomography scan or magnetic resonance imaging of the brain to evaluate for a hypothalamic hamartoblastoma.

Conversely, direct endoscopic evaluation of infants with either multiple midline congenital anomalies or congenital panhypopituitarism may reveal a bifid epiglottis or the presence of a midline laryngeal cleft. This, in turn, may prompt a response that will preclude complications related to airway obstruction, aspiration, and acquired subglottic stenosis.

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DELAYED AND PROGRESSIVE HEARING LOSS AFTER MICROVASCULAR DECOMPRESSION OF CRANIAL NERVES

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An unusual case of unilateral delayed and progressive hearing loss following a microvascular decompression operation on cranial nerves V, VII, and VIII on the left side is reported. Preoperative and postoperative audiologic evaluation revealed a mild high-frequency hearing loss for both ears, normal thresholds for the acoustic middle ear reflex response, and normal brain stem auditory evoked potentials. Three years after this microvascular decompression procedure, the patient noticed slowly decreasing hearing in her left ear, and subsequent serial audiograms revealed a progressive sensorineural hearing loss and a decrease in her speech discrimination score. Brain stem auditory evoked potentials showed progressive changes. Because of the patient's increasing symptoms of vertigo and tinnitus in the left ear, reexploration of the eighth cranial nerve was performed 5½ years after the initial procedure. This second operation revealed reactive tissue around the eighth cranial nerve that was atrophic and yellow. We interpret the delayed and progressive hearing loss to be a result of reactive scar tissue and progressive atrophy of the auditory nerve.

KEY WORDS — brain stem auditory evoked potentials, microvascular decompression, progressive hearing loss.

INTRODUCTION

Microvascular decompression (MVD) of cranial nerves (CNs) in the posterior fossa has been shown to provide a long-term cure for disorders such as trigeminal neuralgia,[1,2] hemifacial spasm,[1] glossopharyngeal neuralgia,[3-5] disabling positional vertigo,[6-8] and tinnitus,[9] where conventional medical therapy has failed. The success rate for trigeminal neuralgia, hemifacial spasm,[1] and disabling positional vertigo[7] is close to 80%. Microvascular decompression has relatively few complications, the most common being a hearing loss of various degrees or complete loss of hearing,[10-12] and, particularly for hemifacial spasm, a hearing loss that could range between 2.5% and 33%.[12]

Injury to the eighth cranial nerve may be caused by stretching of the nerve from retraction of the cerebellum[13] or by a spread of heat from electrocoagulation close to the nerve.[14] However, the use of intraoperative monitoring of auditory evoked potentials has reduced the incidence of hearing loss following MVD.[11]

A hearing loss caused by surgical manipulation usually becomes apparent immediately after the surgical procedure, and the hearing is likely to improve postoperatively. Delayed hearing loss after MVD has been reported in only 3 cases, and its cause could not be determined.[15,16] In the present study 1 patient suffered a delayed and progressive hearing loss 3 years after an MVD procedure on CN V, CN VII, and CV VIII. At reexploration, reactive scar tissue was found around the eighth nerve.

CASE REPORT

A 52-year-old woman had a 3-year history of left-sided atypical trigeminal neuralgia involving the V2 branch of the trigeminal nerve. The pain was constant for several weeks at a time. Conventional therapy with the drugs Tegretol, Dilantin, Lioresal, and Elavil did not alleviate her pain. However, some characteristics of typical trigeminal neuralgia were manifested as well, as the pain usually worsened when eating, talking, washing the face, etc. Three months prior to admission to undergo MVD, the patient noticed a persistent twitching under her left eye, and 3 weeks prior to admission she experienced episodes of true vertigo and constant left-sided tinnitus. The patient was hypertensive and, despite prescribed medications, had marked fluctuations in blood pressure.

Preoperative evaluation revealed normal findings regarding the ears, nose, and throat. There was a mild hypalgesia in the left V2 distribution. An audiogram showed a mild, bilateral, high-frequency hearing loss, more pronounced than expected for the patient's age. Speech discrimination scores were excellent (96% and 96%, respectively), and the thresholds for the acoustic middle ear reflexes (contralateral recordings) were normal, as were the brain stem auditory evoked potentials (BAEPs; Fig 1).

The patient underwent a left retromastoid craniectomy in July 1985 that was performed in the left lateral decubitus position, and via microsurgical techniques the left cerebellopontine angle was exposed.[17] Intraoperative monitoring was performed through-

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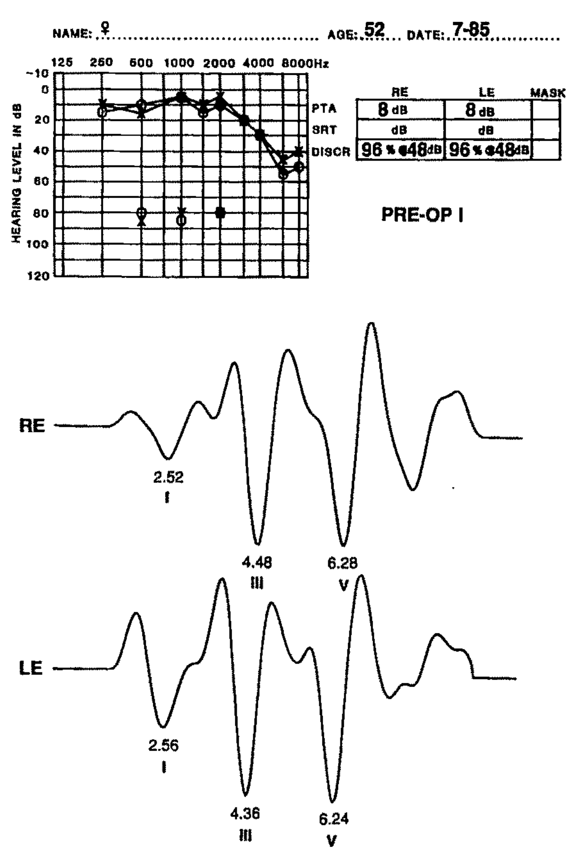

Fig 1. Preoperative audiogram and recordings of brain stem auditory potential (BAEP) in patient with symptoms indicating involvement of cranial nerves V, VII, and VIII, on left side.

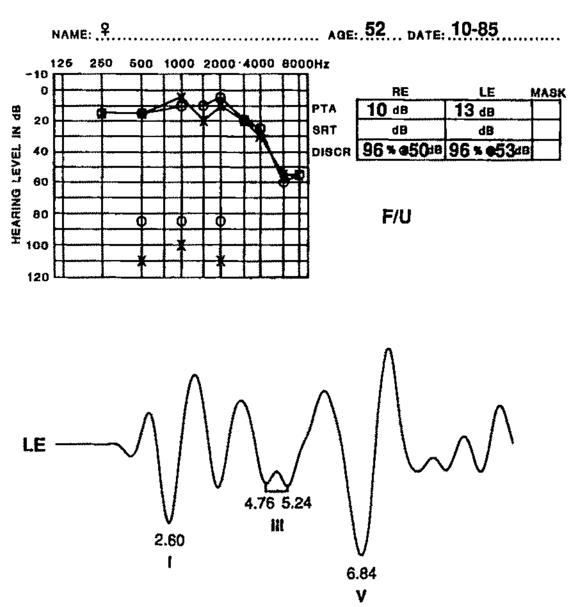

Fig 2. Follow-up audiogram and BAEP recording done 3 months after first operation. Audiogram showed "notch" at 1.5 kHz. Latency of peak V and peak I-V interpeak time interval were prolonged. BAEP recording (from left ear) shows double peak III and subsequent delay of peak V (of 0.6 millisecond).

out the procedure using BAEPs recorded from the scalp and recordings of compound action potentials from the exposed CN VIII.

Findings were as follows. The trigeminal nerve was severely compressed by both the superior cerebellar artery, which was grooving the nerve, and a vein close caudally to the root entry zone. The vein was coagulated, the superior cerebellar artery was mobilized, and three pieces of shredded Teflon felt were placed to hold the artery away from the trigeminal nerve. The flocculus was then elevated and the anterior inferior cerebellar artery was noted to impinge on the root entry zone of the facial nerve, and therefore three pieces of Teflon felt were also placed around (or on) CN VII. Cranial nerve VIII was compressed inferiorly and superiorly by a large dorsal vein, as well as by a branch of the anterior inferior cerebellar artery. When this artery and the cochlear artery were mobilized, four pieces of Teflon felt were placed between the vessels and CN VIII, and the compound action potential recorded directly from the exposed CN VIII changed dramatically. At that point, some of the Teflon felt was removed from CN VIII, after which the compound action potential quickly recovered. The patient's postoperative course was uneventful, except for headaches, the relief of which eventually required a spinal tap. A postoperative audiogram and recordings of BAEPs performed 8 days after the operation showed no change in hearing thresholds, and the BAEPs revealed only a slight increase (0.4 millisecond) of interpeak latencies I-III on the operated side, which is commonly seen after an MVD procedure. The patient's pain totally resolved, as did her vertigo, and her tinnitus had decreased in intensity. A second follow-up audiogram 3 months after the MVD procedure showed a small notch at 1,500 Hz[18] and normal discrimination scores (scores are noted on the audiogram), but the threshold for the acoustic middle ear reflex response was elevated when the left ear was stimulated, indicating involvement of the eighth nerve. The BAEPs recorded from the left side showed a marked increase in the latency of peak III, while interpeak latency III-V was normal (Fig 2). The BAEPs recorded from the right side were normal.

In February 1987 the patient experienced symptoms of imbalance, vertigo, and occasional vomiting that continued to increase in severity. The tinnitus on the left side was still present, but less intensive than it had been prior to MVD. Vestibular tests done at this time revealed a right directional preponderance of 51%, but no spontaneous or positional nystagmus. There was no change in her pure tone audiogram, but the BAEP showed further increased latency of peak III on the left side. The patient was treated with a low dosage of Valium (2 to 4 mg) and Dramamine for her

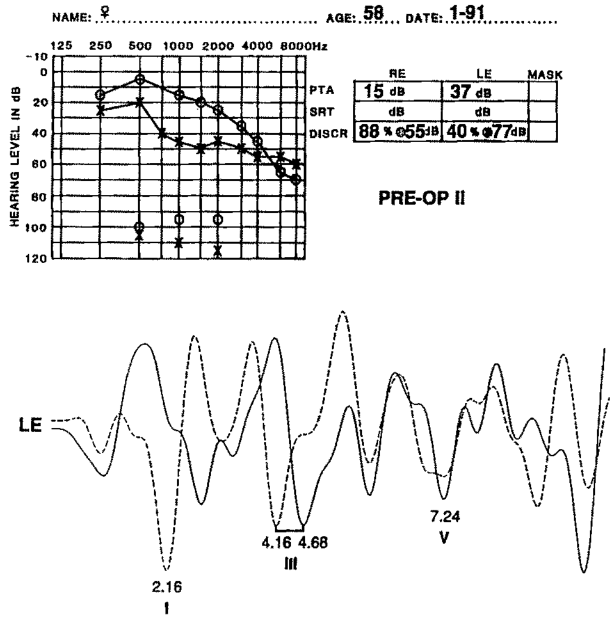

Fig 3. Preoperative audiogram and recordings of BAEP (left side only) done 5½ years after first microvascular decompression procedure. Marked decrease in patient's discrimination score, as well as elevated middle ear acoustic reflex response, was noted. BAEPs (two consecutive recordings) showed marked fluctuations of early peaks.

vertigo and was shortly thereafter able to return to work and to drive a car, etc.

Five years after undergoing MVD, in the spring of 1990, the patient's symptoms of vertigo and left-sided tinnitus began to increase again in severity and she also noticed decreased hearing in her left ear. Serial audiograms showed a moderate sensorineural hearing loss in her left ear (pure tone average of 37 dB) and a disproportionate loss of speech discrimination, which had decreased to only 40% by January 1991 (Fig 3). In addition, her BAEP recorded from the left ear showed progressive changes, with abnormal peak patterns specifically regarding peaks II and III and low amplitude. The BAEP recorded from the right ear was normal. A computed tomography scan showed a dense, enhancing structure in the left cerebellopontine angle, most likely the Teflon pledget that was placed during the first operation in 1985. The acoustic middle ear reflex response was absent when the left ear was stimulated. Because of her symptoms and change in hearing, reexploration of CN VIII was performed in January 1991.

During surgery, reactive tissue, hard as well as rubbery, was found to be covering CN IX and CN X, as well as CN VIII, which was yellow. Some of this reactive tissue was removed, but not all of it, in order to avoid further damage to the CN VIII and the brain stem. The postoperative course was uneventful. A hearing test 3 months later showed an unchanged pure tone threshold and a nonsignificant decrease in discrimination score. The patient's hearing was tested regularly, and a follow-up test 2 years after the second MVD procedure (in October 1993) showed a pure tone average of 57 dB and only an 18% discrimination score—thus, a noticeable decrease in hearing.

DISCUSSION

Hearing loss is a rare complication of MVD procedures, even when the target of the operation is the eighth nerve itself. Møller et al[7] reported a 3.2% hearing diminution or complete loss of hearing in 207 patients who underwent MVD to relieve disabling positional vertigo (a total of 254 procedures). It is presumed that the use of intraoperative monitoring of BAEP has helped to reduce the risk of permanent hearing loss,[11] and such hearing loss after MVD usually occurs immediately after surgery.

Recently some investigators have reported a delayed hearing loss following MVD procedures, but the cause of such hearing loss has not yet been determined. McDonnell et al[15] reported 2 cases of delayed hearing loss following MVD procedures. Schwartz and Gennarelli[16] reported 1 case of delayed hearing loss that occurred 2 days following an MVD procedure to relieve trigeminal neuralgia.

All 3 of these cases had the onset of hearing loss within the relatively immediate postoperative period (within 1 week), whereas the first changes in the pure tone threshold on the operated side of the patient reported on in the present study occurred 3½ years after her initial MVD procedure. It is interesting that changes in the middle ear acoustic reflex threshold and BAEP latencies occurred before any noticeable hearing loss was evident (at 4 months postoperative).

In view of the long delay between MVD and the onset of hearing loss, it is not likely that a direct injury to the eighth nerve during the first operation caused the later hearing loss. The reactive soft tissue that was found to compress the eighth nerve during the second operation might explain the cause of the hearing loss, as it would relate to atrophy of the eighth nerve. The fact that changes occurred in the BAEP before the patient noticed her hearing loss may indicate that the injury to the eighth nerve caused by the reactive soft tissue occurred early (during the initial MVD procedure).

Delayed hearing loss should be considered to be a possible complication of MVD, and thus BAEP should be recorded during such procedures in order to determine whether or not damage to the eighth nerve has occurred. If progressive deterioration of BAEP is noted during such recordings, perhaps reexploration should be done at an earlier stage to possibly avoid further hearing loss and the development of additional scar tissue around the eighth nerve.

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EPITHELIAL HYPERPLASTIC LESIONS OF THE LARYNX

The International Conference on Epithelial Hyperplastic Lesions of the Larynx will be held October 28-30, 1996, in Ljubljana, Slovenia. For more information, contact Bostjan Luzar, MD, or Aleksandar Vodovnik, MD, Institute of Pathology, Korytkova 2, 61105 Ljubljana, Slovenia; telephone 386 61 1403042 (Int. 268); fax 386 61 301 816; E-mail Vodovnik@IBMI.MF.UNI-LJ.SI.

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IMAGING CASE STUDY OF THE MONTH
HEMANGIOPERICYTOMA OF THE MASTICATOR SPACE

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INTRODUCTION

Hemangiopericytoma (HPC) is an uncommon mesenchymal vascular neoplasm that is reported with rare occurrence in the head and neck. These tumors originate from capillary (Zimmermann's) pericytes and can be found wherever capillaries are located, but most commonly arise from musculoskeletal tissues or skin. Generally, they are slow-growing, locally infiltrative, and aggressive, with various rates of pleomorphism and malignant potential.[1-4] Clinical presentations of these tumors are characteristically nonspecific. Radiologic imaging is frequently employed for anatomic delineation, as well as characteristic findings that may provide input into the diagnosis. These findings can be subtle but distinctive. Because the surgical treatment of these lesions involves wide local resection, preoperative knowledge of tumor type can be advantageous to operative planning.

CASE REPORT

A 47-year-old woman presented with a 4- to 5-year history of a slowly enlarging right cheek mass. Over the preceding 6 months, she noted decreased sensation of her right jaw and tongue in the distribution of the third division of the right trigeminal nerve. She reported occasional intermittent right-sided otalgia, tinnitus, and frontotemporal headache. The patient also reported odynophagia for 3 to 4 months but denied dysphagia, dysgeusia, or changes in facial nerve function. The patient had a history of having undergone radiotherapy of the right temporal region for a vascular skin lesion at 2 weeks of age.

Physical examination revealed moderate atrophy of the right temporalis muscle and a smaller than normal pinna. The skin in this area was atrophic and partially discolored. The oropharynx was without mass effect or tongue abnormalities. Fullness of the right infra-auricular region and face was nonspecific, without tenderness or a discrete mass. The neck was without palpable lymphadenopathy. Neurologic examination demonstrated decreased sensation to light touch in the distribution of the third division of the right trigeminal nerve. The first and second divisions were normal. Motor assessment of the masseter revealed normal findings. Her facial nerve function was normal and symmetric (House grade I). The remainder of her cranial nerves were intact.

A computed tomography (CT) scan of the face and neck showed an enhancing right masticator space (anterolateral parapharyngeal space) mass at the mandibular foramen, with compressive erosion changes of the inner table of the mandibular ramus (Fig 1). The initial CT differential diagnosis suggested an aneurysm versus a benign nerve sheath lesion (schwannoma or neurofibroma). Magnetic resonance imaging (MRI) showed a uniformly enhancing, oval soft tissue mass in the right masticator space, intimately associated with the right mandible in the region of the mandibular foramen (Fig 2). The lateral pterygoid muscle was displaced superiorly by the mass. Skull base involvement or extension through the foramen ovale was not seen. The MRI differential diagnosis was limited to V3 (mandibular division of fifth cranial nerve) schwannoma or neurofibroma. An aneurysm was excluded by this MRI appearance. Of interest, the patient also had three other lesions described: a second probable schwannoma of the right Meckel's cave on V2 (maxillary division of fifth cranial nerve) extending into the foramen rotundum, an en plaque meningioma along the dura adjacent to the cavernous sinus (Fig 2) and around the right anterior clinoid, and a small meningioma at the cribriform plate. There were no calcifications or areas of cystic degeneration on either scan.

The patient underwent resection of the mass via a right cervical approach to the masticator and parapharyngeal spaces. The mass measured approximately 3 × 3 × 2 cm in size and appeared to be well encapsu-

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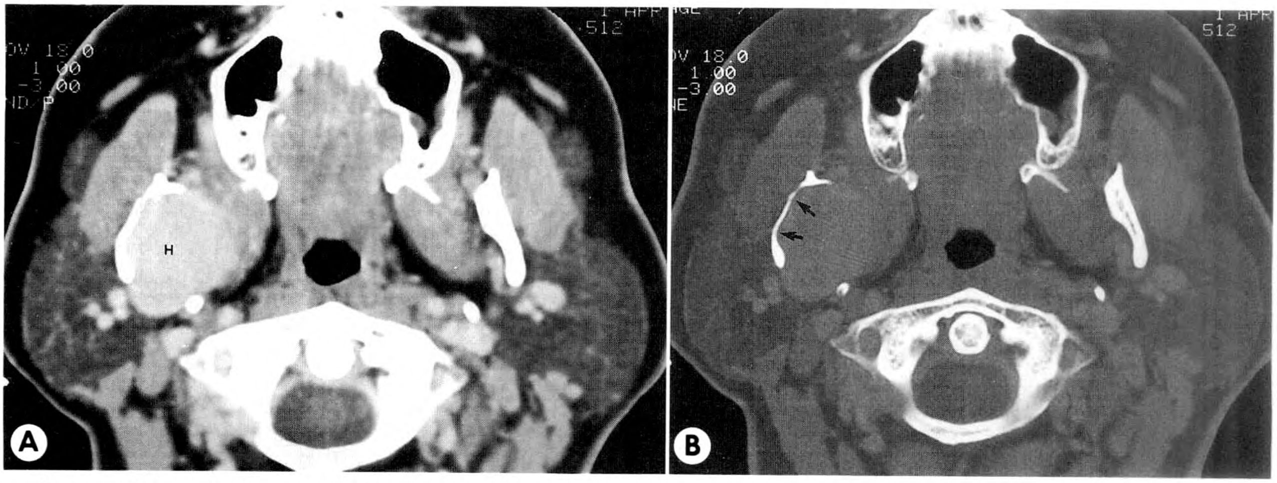

Fig 1. Axial computed tomograms of hemangiopericytoma of masticator space. A) Enhanced, through level of low maxillary sinus, demonstrating avidly enhancing hemangiopericytoma (H) in right masticator space. Mass is nestled between pterygoid muscles and mandible. B) At same level as A, done with bone algorithm and photographed in bone window, demonstrating that mass is remodeling mandible (arrows) with thinning and bowing of bone laterally.

lated and firm to palpation. Two high jugulodigastric nodes that were enlarged were also removed. Pathologic consultation revealed an HPC with a thin pseudocapsule, mild nuclear pleomorphism, and a low mitotic rate. The resected lymph node showed mild follicular hyperplasia and no evidence of metastasis. No areas of hemorrhage or necrosis were noted. Postoperatively, the patient did well, with only moderate trismus. Follow-up MRI at 6 months demonstrated no evidence of residual tumor and no changes in the other lesions.

DISCUSSION

Hemangiopericytoma is an uncommon mesenchymal vascular neoplasm that is reported with rare occurrence in the head and neck. Hemangiopericytomas represent only 1% of adult vascular head and neck tumors. The nasal cavity and paranasal sinuses are the most common sites of presentation in the head and neck.[1-4] The tumor occurs with equal frequency in both sexes and has been found to occur at any age, with a peak in the fifth to sixth decades.[2]

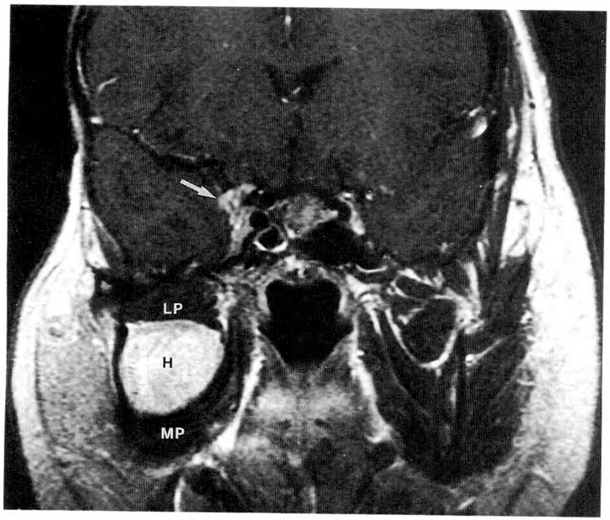

Fig 2. Coronal T1 post-contrast magnetic resonance image through deep face demonstrates hemangiopericytoma (H) splitting lateral pterygoid (LP) above and medial pterygoid (MP) below. Incidentally noted on this same image is probable en plaque meningioma along dura adjacent to cavernous sinus and right anterior clinoid bone (arrow).

This tumor originates from capillary (Zimmermann's) pericytes, which are round or spindle-shaped modified muscle cells with long cytoplasmic processes encircling small blood vessels, thought to give mechanical support to the capillary. Thus, HPCs can be found wherever capillaries are located, but generally arise from musculoskeletal tissues or skin.[1,2] Histologically, the tumors demonstrate pseudoencapsulation. Presence of extensive, uniform vascular channels among densely packed cells and intense reticulin staining of an intercellular meshwork is helpful in distinguishing them from other, more common head and neck soft tissue tumors such as schwannoma or hemangioma.[1,2] They are characteristically slow-growing, locally infiltrative, and aggressive and have various rates of pleomorphism, with malignant and metastatic potential. The cause of HPC is generally regarded as unknown; however, previous trauma, steroid use, and genetic factors have been suggested.[1,2,4] In this patient, there is a history to suggest irradiation as an inciting event. This patient had multiple meningiomas on the same side of her head that received irradiation when she was an infant, as well as a history of a vascular skin lesion.

The clinical presentation of HPC is characteristically nonspecific, with a typical history of a slow-growing, firm, painless mass causing only local symptoms. When pain occurs, it is usually secondary to compression of neurovascular structures, pressure erosion into adjacent bony structures (as in this case), or local soft tissue infiltration.[1-4] Cases of malignant HPC have been described with regional and distant metastasis. Malignancy is ascribed to those tumors with high mitotic rates, necrosis, hemorrhage, increased cellularity, and large tumor size. Metastases occur in half of the patients with malignant HPC. The mode of metastasis is primarily vascular.[2] Because of the vascularity of these tumors, they can demonstrate significant arteriovenous shunting. They can also have associated paraneoplastic syndromes.

Radiologic imaging is employed for anatomic delineation, as well as for input into the diagnosis, which can be subtle. Some authors have described a "typical" CT appearance of HPC in the head and neck as an enhancing, lobulated soft tissue mass with cystic degeneration and solid areas of septation with occasional speckled calcifications.[1] Other authors have found no characteristic features to indicate the pathologic nature of the tumor.[2]

A review of the radiologic literature evaluating HPCs from many anatomic sites revealed consistent CT findings to be hypervascularity with enhancement and well-circumscribed margins, both of which are nonspecific. Central areas of low attenuation representing necrosis or cystic degeneration may occasionally be seen, but again are not specific for HPC, although noted to be suggestive of malignancy if HPC is discovered. The frequency of speckled calcification of these tumors varies greatly, but when present in an HPC, is also felt to be indicative of malignancy. Calcifications, however, are also a common finding among other tumors such as hemangiomas and lymphangiomas.[5-7]

The MRI findings of HPC are consistent with those of any soft tissue tumor, demonstrating increased signal on T2-weighted images and isointensity on T1. Some articles describe HPC with multiple areas of mixed intensity, probably indicating areas of calcification (low intensity) and thrombosis or old hematoma (high intensity).[5,7] It is difficult to distinguish this lesion from other, more common sarcomas[7] and hemangiomas, although some authors feel that the degree of increased T2 signal can be used to differentiate between hemangiomas and HPC.[5] As already well established, CT scan was found to be better at evaluating extent of bony infiltration or erosion, and MRI better at demonstrating tumor margins and perineural involvement.[8] Our patient's scans demonstrated no specific findings, as discussed, and it is likely that this represented evaluation early in the course of this HPC.

Angiography, although infrequently reported among series, has demonstrated characteristic findings, showing a dense, well-circumscribed stain secondary to accumulation of contrast in the tumor's capillary network. The vascular network of HPC is extensive. These findings, however, are not specific to HPC, but are also readily noted in cavernous hemangiomas.[7,8]

The definitive treatment for HPC is wide surgical resection with adequate margins to prevent local recurrence.[1-4] The lack of encapsulation with infiltrating margins is similar to that of a pleomorphic adenoma, and simple enucleation should be avoided.[2] Because of the vascularity of HPCs, preoperative embolization can be employed to minimize blood loss. Integrity of vital structures should be maintained as far as possible. A recent review of the literature found no sufficient data demonstrating effectiveness of adjuvant irradiation or chemotherapy; however, others have suggested that evidence of malignant behavior warrants combined treatment with postoperative irradiation.[1,2] Vigilant postoperative follow-up is indicated to observe for recurrence, which rate is reported to be as high as 50%. Prognosis depends on histologic grading, age, and location of tumor. Increasing aggressiveness is associated with increasing age. A decreased recurrence rate has been noted with tumors in the nasal cavity, which may relate to symptoms and size at presentation.[1]

Diagnostic imaging can be helpful in planning the surgical approach, delineating the extent of tumor spread, and identifying relationships to vital structures. Differential diagnoses can be narrowed or a diagnosis possibly established for particular tumors with classic findings during radiologic examination. This can be done by utilizing radiologically descriptive findings for specific lesions together with a breakdown of anatomic spaces within the parapharyngeal space (parotid, masticator, carotid, parapharyngeal, prevertebral, and paramucosal) in which a tumor can arise. Any preoperative evidence of tumor type can provide an advantage, especially in working in the parapharyngeal space, where generous margins are not easily obtained.

SUMMARY

Hemangiopericytomas are rare tumors of the head and neck. The benign presentation of this tumor belies its high local recurrence rate, local aggressiveness, and malignant potential. In view of these characteristics, workup to provide a diagnosis preoperatively is of significant importance. Diagnostic imaging is helpful in planning operative management, detecting metastases, and narrowing the list of differential diagnoses. However, because of the variety and lack of specificity of radiologic findings, it is generally difficult to provide a diagnosis. A history of a painless, slowly growing, otherwise asymptomatic mass, together with the radiologic findings of a vascular neoplasm, should enhance the suspicion of an HPC as a diagnosis. Hemangiopericytoma should be included in the differential diagnosis of any vascular soft tissue lesion presenting in the head and neck, and plans for surgical intervention should include the possibility of aggressive, wide local resection in order to adequately treat such a lesion should it be encountered.

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CRANIOFACIAL/CLEFT LIP AND PALATE SOCIETY OF GREAT BRITAIN

The next meeting of the Craniofacial/Cleft Lip and Palate Society of Great Britain will be held April 24-26, 1996, in Eghan, Surrey, England. For further information, contact Dr Debbie Sell, Speech and Language Therapy Department, The Hospital for Sick Children, Great Ormond Street, London, WC1N 3JH, United Kingdom; telephone (UK) -71 405 9200 ext. 5043.

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PATHOLOGY CONSULTATION

OROFACIAL GRANULOMATOSIS AND CROHN'S DISEASE

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Orofacial granulomatosis is a clinical entity of either unknown or specific causation. Specific causes include sarcoidosis, chronic infective granulomas, and Crohn's disease. The remainder of cases are idiopathic, but the clinical presentations of orofacial swellings and intraoral mucosal lesions are similar. So, too, are the histopathologic findings of noncaseating granulomas, edema, and chronic lymphoproliferative infiltrate. Crohn's disease is not responsible for all forms of orofacial granulomatosis, but the orofacial manifestations of the disease may herald its diagnosis.

Orofacial granulomatosis is a clinical entity that may or may not have associated systemic abnormalities. The term includes the previously separated eponymic entities of Melkersson-Rosenthal syndrome (MRS) and cheilitis granulomatosis of Miescher.[1,2] The latter is now generally accepted as an oligosymptomatic form of MRS.[2]

It should be understood that orofacial granulomatosis is a descriptive term and has no implication as to a specific cause. Furthermore, orofacial granulomatosis should be nosologically separated from the midfacial necrotizing lesions such as Wegener's granulomatosis and the sinonasal lymphomas.[3] Midfacial necrotizing lesions are principally upper airway diseases; orofacial granulomatosis affects oral and perioral soft tissues — and while mucosal ulcers occur, it is a far less destructive disorder. In each group, chronic specific infective granulomatous disease, eg, tuberculosis, and sarcoidosis are important differential diagnoses. Because of considerable similarities between orofacial Crohn's disease and orofacial granulomatosis, it is recommended that Crohn's disease also be included under the rubric of orofacial granulomatosis and considered in the differential diagnosis.[4,5]

The classic clinicopathologic features of orofacial granulomatosis are, in effect, those described for MRS.[1,2] Clinically, there is recurrent or persistent orofacial swelling, a plicated tongue, and a peripheral facial nerve paralysis. Most patients manifest orofacial swelling in two or more locations, with the lips and buccal mucosa the areas most often involved. Swellings of the palate, gingivae, and sublingual areas can also be seen. The swellings vary in texture from soft, edematous, diffuse, and erythematous to more solid lesions. In the buccal mucosa the swellings assume a lobulated and cobblestone character.

As it is so often with syndromic disorders, not all of the features are considered compulsory or obligatory for the clinical diagnosis of orofacial granulomatosis. Plication of the tongue, for example, is a harmless and rather inconsistent finding: 40% in the study by Worsaae et al.[1] Paralysis of the peripheral seventh nerve is seen in roughly 20% of patients.[1,2]

Histopathologic findings consist of edema in the lamina propria, perivascular mononuclear inflammatory infiltrates, and noncaseating epithelioid granulomas with multinucleated Langhan's type giant cells. Diligence may be required to find the granulomas. In the 33 patients studied by Worsaae et al,[1] granulomas were found in all, but a number of biopsy specimens required serial sections. The granulomas lack specificity for diagnosis, and a 100% yield is not to be always expected.

From a pathogenetic standpoint, it is interesting to note that elimination of odontogenic infectious foci has led to regression or disappearance of the oral and/or labial swellings in some patients.[1]

OROFACIAL CROHN'S DISEASE

Crohn's disease can affect any part of the gastrointestinal tract from the mouth to the anus, and it can be considered as one form of orofacial granulomatosis.

There are several categories of oral lesions in patients with Crohn's disease: 1) macroscopic and microscopic changes also seen in the spectrum associated with gastrointestinal Crohn's disease, 2) nonspecific lesions, 3) lesions related to poor nutrition, and 4) side effects of medication, eg, Stevens-Johnson syndrome.[6] The Table presents the characteristic macroscopic and clinical findings of oral Crohn's disease. Besides the lips and buccal mucosa, lesions may also be found on the gingivae and alveolar mucosa, palate, tongue, and pharynx.[4,6,7]

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MACROSCOPIC FEATURES OF ORAL CROHN'S DISEASE

Diffuse swelling of one or both lips, at times with pain and soreness
Cobblestoning of buccal mucosa (mucosal nodularity with or without linear fissures)
Painful linear or more extensive oral ulcers with hyperplastic margins
Localized facial or lip swellings
Indurated mucosal tags on vestibular or retromolar mucosa

The close similarity of the oral manifestations of Crohn's disease to those of orofacial granulomatosis is also seen in the histopathologic changes in biopsy specimens. Necrotizing granulomas are found in approximately half of the patients' biopsies.[4] Short of formed granulomas, there is a chronic inflammatory cell infiltrate with epithelioid cells and giant cells in the lamina propria and in perivascular locations. Mucosal ulcers, often of a fissure type, edema, and nodular lymphoid aggregates, like those found in the gastrointestinal tract, complete the histopathologic picture.

In documented cases, oral lesions may coincide with, precede, or follow a diagnosis of enteric involvement.[4,6,7] In a review of reported cases, Talbot et al[6] found that approximately one third of patients had oral lesions that antedated abdominal symptoms or diagnosis of intestinal Crohn's disease. The remainder had the onset of oral lesions after abdominal evidence of their disease. If there are other extraintestinal manifestations of Crohn's disease, there is an increased likelihood of oral lesions appearing.[6]

Although nonspecific aphthous stomatitis occurs with a frequency of up to 20% at some time during the course of Crohn's disease, it is not considered as a reliable indicator of oral involvement by the disease. Nonetheless, the aphthae associated with Crohn's disease tend to be more widespread and severe, and may occur with, or precede, a symptomatic exacerbation of the intestinal disease. In general, however, exacerbation of the orofacial disease is not usually associated with disease activation in the bowel.[6] It has been also noted that colonic, rather than small intestinal, Crohn's disease is more often associated with oral manifestations.[6]

Arguments against including oral Crohn's disease as an etiologic basis for some patients with orofacial granulomatosis are few. To say that orofacial granulomatosis, exclusive of sarcoidosis or specific infective granulomas, is only a manifestation of Crohn's disease is, however, too exclusive. Not all patients with oral granulomatosis manifest intestinal disease, and to consider it a *forme fruste* disorder ignores clinical laboratory evidence. The enigmatic facial nerve paralysis component of orofacial granulomatosis is not seen with Crohn's disease, and the presence of a serum antibody to mycobacterial 65 kd stress protein, found in oral Crohn's disease, in not found in non–Crohn's disease orofacial granulomatosis.[8]

Various treatments have been used for oral Crohn's disease. These have included topical, intralesional, and systemic medications. Williams et al[4] indicate that in their experience, the only satisfactory treatment has been systemic corticosteroids. This improves the oral ulcerations and swelling of the lips, but is less effective for the buccal mucosal cobblestoning. Oral surgery is not warranted because of the recurrences of the oral lesions and the lack of proof of benefit.[6]

Patients without enteric disease may show a spontaneous resolution or may show some degree of improvement with intralesional steroids. The recorded good responses to elimination of chronic oral or odontogenic infection mandate that such foci always be treated.[1]

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BOOK REVIEWS

Practical Approach to Head and Neck Tumors

Jack Gluckman, Patrick Gullane, and Jonas Johnson. Hard cover, indexed, 212 pages, 1994. Raven Press, New York, NY, $75.

The purpose of this small volume is to present a practical management philosophy as advocated and conveyed by three head and neck surgeons who are actively involved in the day-to-day management of patients who have cancer of the head and neck. Utilized is a no-nonsense diagnosis-treatment approach by each author outlining his approach and differences in the approach between authors. There are no illustrations, diagrams, or radiographs. This book would be useful to the individual practitioner who is a "several times per year" surgeon of head and neck lesions and who utilizes references of journal articles and book chapters, but yet would need a different and more practical, informative approach to certain aspects of diagnosis and treatment.

The authors have handpicked the topics, and there are 15 of them. The usual ear, nose, and throat subjects are included, along with the lip, skin, multiple primary tumors of the upper aerodigestive tract, and "incurable" head and neck cancer. Sections are well structured, with an introduction typically giving incidence for the particular lesion. Anatomy, staging, patient evaluation, treatment management, and diagnosis are typically discussed.

In general, a book of this type cannot give extensive coverage to all aspects of the subject. This one, unique as it is, does contain crucial basic information, although it is the opinion and experience of one to three clinicians.

Dennis G. Pappas, MD
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Vandals at the Gates of Medicine. Historic Perspectives on the Battle Over Health Care Reform

Miguel A. Faria, Jr. Hard cover, illustrated, indexed, 403 pages, 1994. Hacienda Publishing, Macon, Ga, $41.95.

The aim of the author is to convey through historical precedents in medical history and medical ethics the reintroduction and reimplementation of free-market, ethical, patient-oriented medical care in both academic and private settings. In doing so, Dr Faria touches on the origin, development, and implications of the ethics of the medical profession from its beginnings in time. These ethical disciplines are interconnected with various present health care topics of today. Solutions are conveyed.

According to the author, one must focus on three areas, to be influential in reintroduction and reimplementation of the free medical market. First, the physician must communicate to the community as a whole, to explicate the realities of the health care delivery system (explaining the pitfalls of controls, bureaucracy, price fixing, and lack of free market competition). Second, the physician must communicate to the patient the reasons for spiraling health care costs (defensive medicine, cost of high technology, etc). Third, the physician must communicate to the patient the advantages of preventive medicine, because the cost of health care and preventive medicine are interrelated.

Dr Faria synthesizes and extends the earlier books and articles published on health care debates. His presentation is certainly very persuasive. His knowledge of the history of civilization is spectacular. He delves into prehistoric times, ancient Mesopotamia, Greece, Rome, the Dark Ages, and the Renaissance, all the while providing pertinent and applicable material recounting the trials and tribulations of the clinician physician.

His perspective of medical history may be accurate, but he too heavily relies on recent medical histories to convey his story; eg, part 1 includes 47 bibliographical references, 32 of which are as recent as the 1980s. Only Francis Adams' translation of Hippocrates' works is referred to from the period of the 19th century. Instead of referencing George Santayana's "those who cannot remember the past are condemned to repeat it" from the source, it is taken from a book of quotations. The symbol of medicine — the caduceus (two snakes) or the staff of Aesculapius (one snake) — should have been addressed from the 1958 article by Frederick Stenn ("The symbol of medicine." Quarterly Bull Northwestern Med School 1958;32:74-87) — a very scholarly thesis and one that is very convincing on this subject.

One cannot succeed unless we understand the rules. Dr Faria, at his best, advises that physicians be proactive rather than reactive in health care policy matters — that we (physicians) have a duty to the public and to ourselves to be well versed in health care and be able to discuss or debate crucial health issues with the media, health care experts, attorneys, business leaders, and politicians. In his view, physicians must sharpen their rhetorical abilities and writing skills to write effective editorials and letters to newspaper and magazine editors so they can reach the public. Granted, that is easy to say, less easy to execute. However, Dr Faria defines a problem.

Students and seasoned practitioners should have an interest in the socioeconomic information conveyed in this volume. It is also an accomplished addition to the history of medicine.

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PEDIATRIC EXERCISE-INDUCED LARYNGOMALACIA

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Laryngomalacia is a well-recognized cause of airway obstruction and inspiratory stridor in infants. As children grow and become more active, laryngomalacia may manifest in different, unexpected ways. Otherwise healthy athletes may generate enough inspiratory force to draw the aryepiglottic folds into the endolarynx, causing a subtotal glottic obstruction. This problem may be overlooked or attributed to asthma, lack of fitness, or functional abnormalities. The purpose of this report is to review the prevalence, diagnosis, and treatment of exercise-induced laryngomalacia (EIL) in children and young adults. To study the incidence and diagnosis of this disorder, we examined 10 healthy volunteers. Fiberoptic laryngoscopy was used to videotape each subject's larynx during active exercise on a stationary bicycle. All volunteers demonstrated altered laryngeal dynamics with exercise, and 1 of the 10 volunteers developed laryngomalacia. Anatomically, it appears that the aryepiglottic fold serves as the critical point of obstruction. When symptomatic, laryngomalacia may be treated with supraglottoplasty. We have had experience with 2 EIL patients in the last 12 months who have undergone carbon dioxide laser microlaryngoscopy with supraglottoplasty. Both patients benefited significantly from surgery. We conclude that EIL is underdiagnosed but responds well to treatment.

KEY WORDS — exercise, laryngomalacia, larynx.

INTRODUCTION

Laryngomalacia is the most common cause of stridor in the first year of life. Typically, symptoms are benign, presenting at or shortly after birth and abating within 1 to 2 years.[1,2] Approximately 10% to 20% of affected infants have obstructive apnea, feeding difficulties, cyanosis, or failure to thrive requiring surgical intervention.[3,4] An endoscopic supraglottoplasty, when necessary, usually alleviates troublesome symptoms.

As children become more physically active, laryngomalacia may present in different, unexpected ways. Certain older pediatric athletes may generate enough inspiratory force to draw the aryepiglottic folds into the larynx, partially obstructing the glottis. We have identified a subset of patients who experience this form of laryngomalacia only during exercise, and have termed this condition exercise-induced laryngomalacia (EIL).[5] Although EIL is not widely recognized, a large number of people may be affected, particularly in our fitness-oriented society. Very little is known about exercise physiology involving the larynx in general or EIL in particular. The purpose of this report is to review the prevalence, diagnosis, and treatment of EIL in children and young adults.

METHODS

Healthy university students were recruited to study laryngeal function during exercise. Anyone with a history of asthma or lung or heart disease was excluded. All participants had normal findings on resting laryngeal examination. Each subject had baseline pulmonary function testing, including functional vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1). Flow-volume loops also were obtained.

After applying topical nasal Xylocaine, a member of the investigative team inserted a transnasal flexible fiberoptic laryngoscope. The subject then began exercising on an incremental cycle ergometer (exercise bicycle). The work rate was increased by 30 to 40 W each minute until the subject's heart rate reached 85% of the predicted maximum ([220 – age in years]/minutes). The larynx was videotaped throughout each workout (approximately 10 minutes). Specific observations were made of supraglottic dynamics before and during exercise. Data also were obtained on endolaryngeal function during expiration versus inspiration. Each participant repeated pulmonary function testing during and/or immediately after exercise.

RESULTS

Ten volunteers were recruited. There were 6 males and 4 females, with an age range of 16 to 32 years (mean, 23.6). No patients had stridor or airway distress. The post-exercise FEV_1/FVC ratio increased

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REPRINTS — John P. Bent III, MD, Division of Otolaryngology, BP-4154, Medical College of Georgia, Augusta, GA 30912-4060.

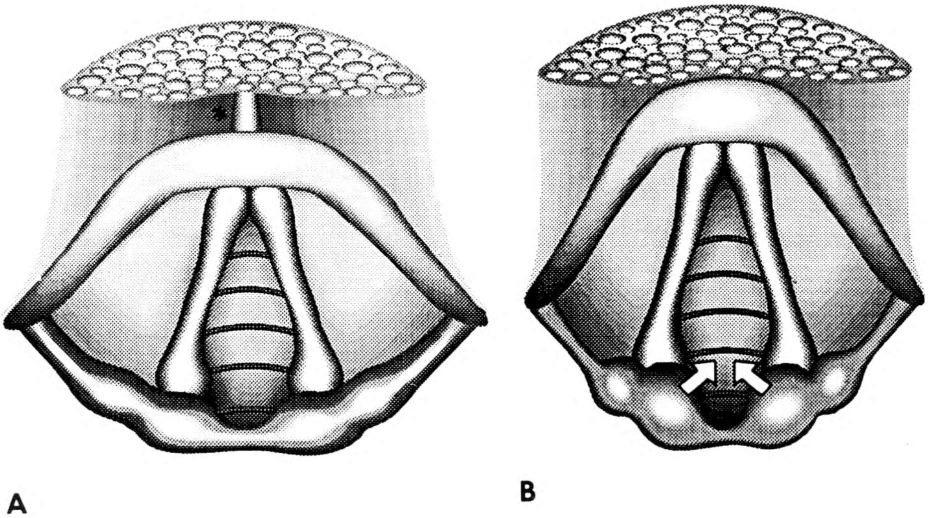

Fig 1. A) Normal larynx at rest during inspiration. Asterisk — hyoepiglottic ligament. Larynx has elliptical shape. B) Inspiratory phase of normal laryngeal response to exercise, 85% maximal heart rate. Note anterior movement of epiglottis relative to position in A with obliteration of vallecula. Larynx has assumed diamond shape with thinning of aryepiglottic folds. This adds relative mass to corniculate cartilages, which may prolapse into glottis (arrows).

≥10% in 5 of 10 patients and decreased in none. Flow-volume loops were obtained in 8 patients; the post-exercise inspiratory curve showed no change in 5, slight flattening in 1, and significant increases in 2 subjects.

Because of patient and endoscope movement, changing expiratory and inspiratory time, gagging, straining, and swallowing, precise and reproducible measurements of glottic size could not be obtained. Laryngeal lumen area also could not be calculated, since the endoscope only provides a two-dimensional view. However, several generalizations could be made. The glottis could be seen to dilate during exercise in both inspiration and expiration, changing from an elliptical to a diamond shape. In all volunteers the vocal folds adducted during restful expiration, but moved to a fixed abducted position during heavy exertion. It also appeared that inspiratory glottic size remained slightly larger than expiratory size even during maximal exercise. At maximal exertion, the vocal folds appeared to elongate during inspiration, possibly because of tensing of the cricothyroid muscle, further augmenting the endolaryngeal area.

Precise measurements of the supraglottic lumen could not be made. However, in 8 of 10 subjects, the epiglottis clearly rotated anteriorly on inspiration, flattening against the tongue base and obliterating the vallecula (Fig 1). The two flow-volume loops with increased inspiratory area were both recorded by subjects with an anteriorly rotated epiglottis. Five patients (4 of whom had an anteriorly rotating epiglottis) had aryepiglottic folds that seemed to thin and elongate, as if under tension, giving the folds an appearance of decreased mass.

Two patients (both having an anteriorly rotated epiglottis and aryepiglottic thinning) developed a tendency for the corniculate cartilages or arytenoid mucosa to move anteriorly and inferiorly with inspiration on extreme exertion. In 1 case this actually progressed to slight glottic obstruction, but no stridor developed. This particular patient was not well conditioned, and could not sustain prolonged maximal heart rate. Unfortunately, no flow-volume loop was obtained in this patient with apparent laryngomalacia. The other patient with posterior supraglottic instability was the only volunteer to have a decreased inspiratory curve on post-exercise flow-volume loop. Both of these 2 patients were female.

CLINICAL EXPERIENCE

In 1984 Lakin et al[6] described a 32-year-old female jogger who had had exercise-induced stridor since college. Although the authors observed findings compatible with EIL, they attributed the problem to rhinitis and reported some improvement with medical therapy. A second case, involving a 16-year-old girl who developed inspiratory stridor and dyspnea while playing basketball, was reported in 1994.[7] Exercise-induced laryngomalacia was diagnosed by using flexible laryngoscopy to observe tissue in the arytenoid region prolapsing medially during inspiration, a phenomenon that only occurred as the patient exercised. This patient chose speech therapy over supraglottoplasty, and it resulted in improvement, but upper airway obstruction persisted during prolonged exercise. Both of these cases were initially misdiagnosed as exercise-induced asthma (EIA).

We have now had experience with 2 EIL patients who have undergone endoscopic supraglottoplasty. The first surgically treated patient was a 10-year-old girl with a 1½-year history of worsening exercise-induced dyspnea initially misdiagnosed as EIA.[5] Exercise-induced laryngomalacia was diagnosed after flexible endoscopic exercise testing. Pre-exercise and post-exercise flow-volume loops demonstrated a variable extrathoracic compression induced by exer-

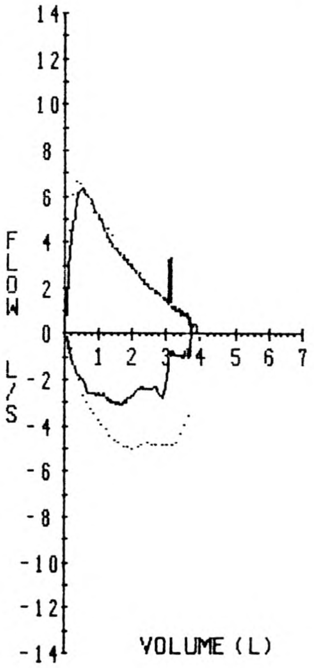

Fig 2. Pre-exercise and post-exercise flow-volume loop showing variable extrathoracic obstruction that developed after exercise. Dotted line — pre-exercise, solid line — post-exercise.

cise (Fig 2). Repeat exercise testing after supraglottoplasty showed complete resolution of the problem, and the patient was able to participate in competitive athletics without any airway symptoms (Fig 3).

The second patient with EIL was a 10-year-old boy who, like the first patient, had no history of chronic illnesses or airway problems earlier in life. He presented to the pediatric otolaryngology clinic with a 12-month history of stridor and dyspnea with exercise. The problem had begun the preceding summer at camp, and seemed to be exacerbated by upper respiratory tract infections. One severe episode of airway obstruction required emergent medical attention, but resolved after treatment with racemic epinephrine. He initially was treated with bronchodilators without success. While sedated for bronchoscopy, he was observed to have collapse of his posterior supraglottic mucosa into the larynx. This episode of laryngomalacia created inspiratory stridor and reproduced his exercise symptoms. Given the patient's history and bronchoscopy findings, we made the diagnosis of EIL. Flexible laryngoscopy confirmed adequate vocal fold mobility and demonstrated posterior supraglottic laryngomalacia on deep inspiration. Flexible endoscopic exercise testing was not done, as this was an anxious child who was unwilling to cooperate for the test. A supraglottoplasty was performed with the carbon dioxide laser and suspension microlaryngoscopy. The corniculate region of the aryepiglottic fold was excised bilaterally, and 6 months postoperatively he is dramatically improved.

DISCUSSION

Thomson and Turner realized almost 100 years ago that there were several different anatomic sites of laryngomalacia: "...we have noticed three main types: 1. ... the sides of the opening were sucked together to form a mesial slit..., 2. ...the epiglottis was drawn backward and downward to act as a lid..., 3. the arytenoid cartilages met in the midline."[8(p1562)] Their observation remains relevant in modern otolaryngology, although in the third subtype it is actually the

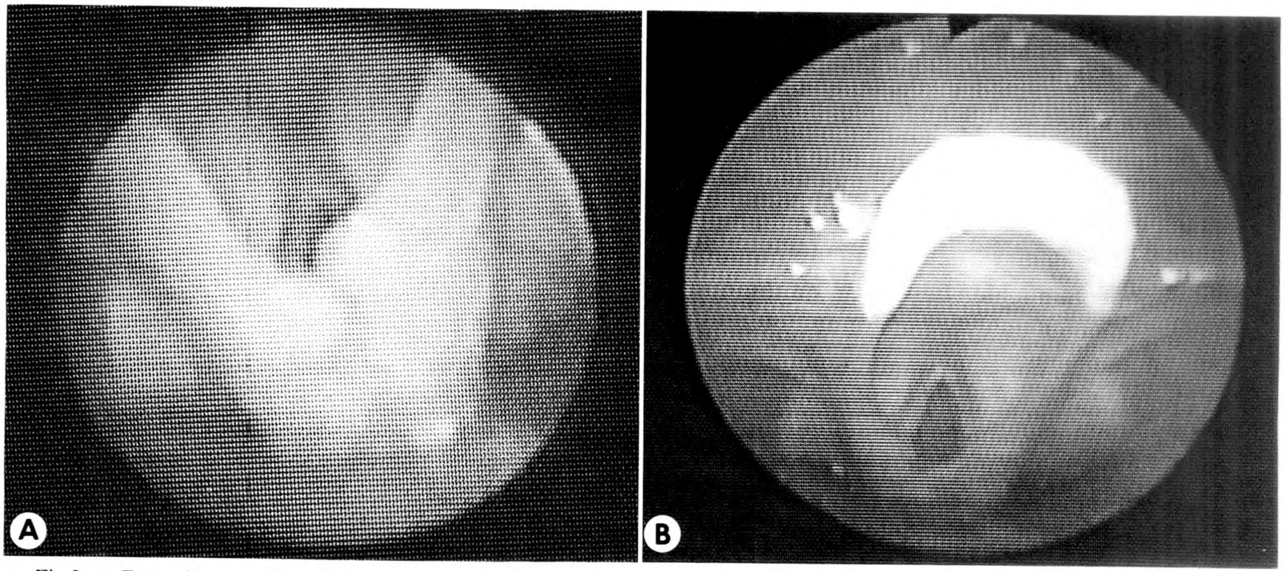

Fig 3. A) Extensive exercise-induced laryngomalacia with almost complete obstruction of glottis during inspiration. Aryepiglottic folds have thinned, giving corniculate cartilages appearance of increased mass. B) Same patient during inspiration (85% maximal heart rate), 3 months after endoscopic supraglottoplasty. Supraglottis has opened considerably.

posterior aryepiglottic fold that rotates into the endolarynx to create the obstruction. Since the advent of fiberoptic instruments, physicians have been able to diagnose laryngomalacia with much greater reliability, yet the cause of laryngomalacia remains a source of controversy and confusion. Infant cadaver studies have shown that the vocal cords are the least compliant region of the upper airway.[9] By Bernoulli's law, transluminal pressures will be greatest in this narrow, noncompliant space. During extreme exertion the relative contribution of the upper airway to total pulmonary resistance increases,[10] and as higher negative translaryngeal gradients develop, inspiratory collapse of a poorly supported supraglottis can occur. Although increasingly negative inspiratory force predisposes to EIL, other factors must be involved in the small subset of patients who experience this problem.

Laryngomalacia has often been attributed to immature laryngeal cartilage; however, this theory has not been effectively or consistently supported by histologic investigations. In 4 children who died of laryngomalacia, Kelemen[11] found the aryepiglottic folds void of muscles, although similar findings also were observed in controls. In 1 of these children, he noted that the epiglottic cartilage was unusually thin, but this was not observed in the other patients. Abnormally hypercellular cartilage was reported by Shulman et al[12] in 2 children with suspected familial laryngomalacia. However, 1 of these children had been intubated for approximately 10 weeks, and both histologic sections were taken from the trachea, not the larynx. Zalzal et al[2] reported that 9 of 10 cartilage specimens removed from patients with laryngomalacia were histologically normal; the 1 atrophic cartilage specimen could not be explained. Templer et al[13] also reported histologically normal cartilage after performing a supraglottic laryngectomy for laryngomalacia. They hypothesized that laryngomalacia was due to poor attachment, rather than a defect in the cartilage.

Some evidence suggests that laryngomalacia results from abnormal supraglottic neuromuscular tone. If neuromuscular tone is decreased in laryngomalacia, it may be a result of central nervous system dysfunction. Prescott's[14] review of 40 laryngomalacic children who required "epiglottoplasty" revealed a 30% incidence of neurologic disorders. Apley's[15] report that 43% (19/44) of infants with laryngomalacia eventually developed mental retardation suggests a strong correlation with neurologic impairment. Belmont and Grundfast[16] noted an 80% incidence of gastroesophageal reflux in 20 children with congenital laryngeal stridor, leading them to suggest that the connection between congenital laryngeal stridor and gastroesophageal reflux may be one of shared neuromuscular immaturity. That induced laryngomalacia has been observed during bronchoscopy in sedated children[17] suggests that central inhibition of laryngeal neuromuscular tone may critically alter laryngeal dynamics. The fact that acquired laryngomalacia has been reported in patients who have suffered drug overdose, cerebral palsy, closed head injury, meningitis,[18] and stroke[19] supports the connection between central nervous system insults and laryngomalacia.

Lesions that narrow the airway predispose to acquired laryngomalacia, although anatomic abnormalities, such as "infantile" or "omega-shaped" epiglottises, are not a sine qua non of the diagnosis and occur with equal frequency in healthy persons. Kletzker and Bastian[20] reported 3 patients with goiter, bilateral vocal cord paralysis, and laryngeal edema secondary to bilateral radical neck dissections, respectively, who presented with acquired laryngomalacia. We recently treated a 5-year-old girl with Cotton grade I subglottic stenosis, left vocal fold paralysis, and right vocal fold paresis who presented with exercise-induced dyspnea and stridor. As she became more active, she developed disabling stridor and dyspnea while at play. Flexible laryngoscopy revealed laryngomalacia of the posterior supraglottis with maximal inspiratory effort. Because of her stenosis, transglottic pressures were created during exertion that exceeded her supraglottic strength. Although her symptoms occurred during exercise, we did not consider her to have EIL. The laryngomalacia was most likely secondary to increased airflow through her coexisting airway lesions, rather than changes specifically related to exercise.

Otherwise healthy individuals who present with EIL in late childhood must be differentiated from children with persistent congenital laryngomalacia. Smith and Cooper[21] studied 20 children (mean age of 8 years) who had congenital laryngomalacia. Thirty percent (6/20) had variable extrathoracic obstruction on flow-volume loops,[21] suggesting that some cases of idiopathic late-onset laryngomalacia represent congenital laryngomalacia that was previously overlooked owing to minimal symptoms. In another series 15% (3/20) of congenital laryngomalacia patients had inspiratory stridor associated with exercise over 8 years after initial diagnosis.[22] In the cases of EIL we have described, the absence of congenital stridor was specifically documented. The lack of neonatal stridor separates EIL from the occasional case of congenital laryngomalacia that may persist as exercise-induced airway obstruction.

Exercise-induced laryngomalacia has its basis in laryngeal dynamics during maximal airflow. A limited amount of knowledge exists regarding laryngeal exercise physiology, and there are no clear answers to explain why a previously healthy person would develop laryngomalacia during exercise. It is generally accepted that the glottis, while at rest, narrows with expiration and opens with inspiration. Two studies have examined the larynx for changes in normal breathing patterns induced by exercise. Both demonstrated that exercise causes glottic dilation in both phases of respiration. Hurbis and Schild[23] compared the effect of exercise on 15 controls and 15 EIA patients. Laryngoscopy was performed for 10 respiratory cycles before, during, and after 8 minutes of exercise, but continuous monitoring was not done. They found that exercise caused an increase in glottic area during both inspiration and expiration, although the effect was greater on expiration. The only exception applied to patients with severe EIA, who had a statistically significant lack of expiratory dilation with exercise. England and Bartlett[24] examined the laryngeal response to both exercise and hyperpnea among 5 healthy volunteers. They also found that exercise caused a biphasic increase in glottic area and, in particular, reduced the extent of normal glottic narrowing during expiration. The effect of hyperpnea was virtually identical to that of strenuous exercise. As in Hurbis and Schild's study, continuous laryngeal videotaping was not performed. A different study reported that hypercapnea caused a slight decrease in laryngeal resistance, but this finding was not correlated with laryngeal endoscopy.[25] The observation that patients with tracheotomies close their hypopharynx to airflow has led to the suggestion that the larynx responds to the amount of tidal volume.[26] Whether the laryngeal response to exercise is triggered by primitive reflexes, a chemically mediated change in neuromuscular tone, or simply increased airflow is a matter of speculation.

Our study is the first to continuously view the larynx throughout the entire exercise period. The uninterrupted visual monitoring permitted confirmation of the two previous reports that observed laryngeal dilation during exercise. Laryngeal dilation during expiration was documented by a >10% increase in the FEV1/FVC ratio in 5 patients. We also had a unique opportunity to hypothesize on the supraglottic response to exercise. In the majority of subjects (80%), the epiglottis rotated anteriorly onto the tongue base, an apparent adaptation to reduce supraglottic turbulence. The anterior movement of the epiglottis also may be a physiologic adaptation to facilitate mouth breathing. Belmont and Grundfast's[16] dissection of 10 adult cadavers clarified the muscular actions within the supraglottis. On the basis of their diagrams, it appears that the following three muscles would most likely tilt the epiglottis forward: the stylopharyngeus muscle, which forms the pharyngoepiglottic fold and inserts into the aryepiglottic fold; the palatopharyngeus muscle, which inserts into the pharyngoepiglottic fold with the stylopharyngeus muscle; and the hyoglossus muscle, which inserts into the hyoepiglottic ligament, giving it the capability to exert anterior traction on the epiglottis.[16] As these muscles pulled the epiglottis forward, the aryepiglottic fold, at least in some of our volunteers, lengthened and thinned. The thinned aryepiglottic fold appeared to have less mass during exertion than at rest, causing the corniculate and cuneiform cartilages to acquire a relative increase in mass. Two of our study subjects responded to exercise in this fashion, and also had slight prolapse of the corniculate cartilages and adjacent mucosa into the posterior glottis with inspiration (Fig 1B). The maintenance of posterior supraglottic support in the other 8 subjects (with and without aryepiglottic fold thinning) suggests that they had more effective muscular support, better neuromuscular tone during exercise, a greater absolute cross-sectional area of the airway, or a combination of these factors.

The 1 patient who appeared to be developing EIL did not have the cardiovascular fitness to sustain exercise. However, it was our impression that with continued energy expenditure, she would develop symptomatic upper airway obstruction. Although this patient was a non-athlete, our clinical experience indicates that patients presenting with EIL tend to be physically active. One may hypothesize that highly conditioned athletes, who can generate more airflow for longer durations, may be at greater risk for EIL. Female sex also may be a risk factor for EIL. Both subjects with posterior supraglottic weakness, as well as 3 of the 4 reported patients with EIL, are females. Females have smaller larynges than males, and at equal rates of airflow, collapse is more apt to occur than in males.

The actual prevalence of EIL is unknown, but our clinical experience and investigations suggest that it may affect a large number of active people. Weiler et al[27] reported that 12% to 14% of college athletes had exercise-induced respiratory complaints. Medical evaluation of these patients usually focuses on bronchial hyperreactivity, but often no objective abnormalities are detected.[28] Laryngomalacia is unlikely to be considered in the differential diagnosis, so that patients with actual disorders are apt to pursue a sedentary lifestyle. Persons with undiagnosed EIL who persist in athletics may be regarded as poorly conditioned, or worse, as malingerers. Another study of 983 college athletes found the incidence of EIA to be 2.8%.[29] An equivalent number of athletes complained of exercise-induced dyspnea without objective abnormalities. Since the larynx was not monitored, it is conceivable that some of these athletes had EIL.

In order to meet the diagnostic criteria for EIL, airway obstruction should develop during extreme exertion and occur primarily during inspiration. A careful history should alert the physician to the possibility of EIL. If the patient reports a history of congenital stridor, persistent laryngomalacia, rather than EIL, should be considered. Also, EIL does not produce wheezing and should not improve with bronchodilators.

Several tests must be considered if the physician suspects EIL. Methacholine challenge may reveal EIA even if findings on exercise testing are normal.[30] The examiner should be alert to the possibility of paradoxical vocal cord motion,[31] which also may present during exercise.[28] Resting laryngoscopy findings should be entirely normal. Exercise testing confirms the diagnosis if stridor and dyspnea occur simultaneously with the observed laryngomalacia. Post-exercise flow-volume loops should reveal a reduced inspiratory area. This is unlike asthma, in which the expiratory component of pulmonary function tests is reduced. In younger children who will not cooperate with exercise laryngoscopy, post-exercise flow-volume loops may be the only means of differentiating EIA from EIL.

After EIL is confirmed, supraglottoplasty can effectively reduce the redundant supraglottic tissue. Of the 2 postoperative patients, 1 is now asymptomatic and the other is significantly improved. In the former case postoperative exercise testing confirmed removal of the aryepiglottic tissues that had previously prolapsed into the larynx, as well as improved airway dynamics.[5]

CONCLUSIONS

Exercise-induced laryngomalacia is an unheralded and unique subtype of laryngomalacia. It involves a set of physiologic changes in supraglottic dynamics, as demonstrated by our volunteer population. The occurrence of EIL probably requires a combination of high airflow, anatomic variants, and/or decreased supraglottic neuromuscular tone. The critical site of obstruction appears to be in the posterior supraglottis. Proper diagnosis requires appropriate history and exercise-induced inspiratory obstruction documented with flexible laryngoscopy or flow-volume loops. In carefully identified patients supraglottoplasty appears to be effective.

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ENDOSCOPIC LASER-ASSISTED RESHAPING OF COLLAPSED TRACHEAL CARTILAGE

A LABORATORY STUDY

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Repair of anterior tracheal wall collapse is a common and troublesome problem encountered by the head and neck surgeon. The standard treatment calls for an open procedure with or without stenting, depending on the extent of the damage. To avoid the morbidity of the open procedure, a new concept of endoscopic cartilage reshaping was investigated in a laboratory animal study. It involved the application of a 1.44-μm pulsed neodymium:yttrium-aluminum-garnet (Nd:YAG) laser at relatively low power to restructure without devitalizing cartilage. An in vivo study was done in six dogs to determine appropriate laser dosimetry in a model of tracheal wall collapse created by a tracheotomy. The deformed cartilage was treated endoscopically with a noncontact 1.44-μm Nd:YAG laser, at 2 to 4 W of power with a repetition rate of 20 Hz, in three animals. As a control, three animals had endoscopic cartilage incisions followed by stent placement. Six weeks postoperatively, both groups had an adequate airway lined by healthy mucosa. In the animals with stenting, however, there was stenosis formation due to scarring at both ends of the stent, with significant inflammatory response in the local area. This study shows that it is possible to use low-power laser energy to reshape cartilage without destroying its viability, and to restore the tracheal wall to a normal contour without ablation or vaporization. The reshaped cartilage will tend to retain its shape with functional elastic force, as seen in in vitro studies. These preliminary results are encouraging, and it seems reasonable to consider using the technique in selected clinical cases as an alternative to conventional open surgery.

KEY WORDS — cartilage reshaping, neodymium:yttrium-aluminum-garnet laser, tracheal wall collapse.

INTRODUCTION

There are many causes for anterior tracheal wall collapse. The most frequent are improper or high tracheotomy, traumatic intubation with cartilage fracture and subglottic edema, infection around the tracheotomy site, and excessive movement of the tracheotomy tube in patients on ventilatory support. Anterior tracheal wall collapse has been cited as a major cause of delayed tracheotomy decannulation in infants. Nearly 10% of infants with long-standing tracheotomies had severe peristomal tracheomalacia and/or significant cartilage collapse.[1] The usual surgical repair utilizes a choice of traumatic procedures including re-creation of a lower tracheotomy, ablation of scar tissue, and placement of a stent for various periods of time.[2] These open techniques are often associated with a high rate of morbidity, and prolonged stenting can aggravate the ongoing tracheal inflammation and serve as a potential source for a new stenosis.[3]

A new concept, called laser cartilage shaping, has been proposed and its effectiveness demonstrated in reshaping rabbit's ear cartilage in in vitro and in vivo experiments.[4-6] The in vitro laboratory testing showed that the carbon dioxide (CO_2) laser used by previous investigators could not produce a similar effect in tracheal cartilage and forced us to look for another laser with deeper penetration into biologic tissue. We selected the 1.44-μm pulsed neodymium:yttrium-aluminum-garnet (Nd:YAG) laser for reshaping cartilage from fresh canine tracheal rings.[7] These arc-shaped cartilages were irradiated with the laser at 2 W of average power (0.1 J per pulse at 20 Hz) in noncontact mode with a spot of approximately 3 mm moved over the exposed surface in a painting-like fashion with a velocity of 1.5 to 2.0 mm/s. The irradiation lasted until the cartilage either became straight or developed an inverted curvature (5 to 6 minutes). The reshaped cartilage specimens retained their new shape and maintained nearly constant elastic force for up to 11 days after irradiation. The in vitro experimentation helped to develop and test combinations of laser parameters that were then used in an in vivo animal study to explore the feasibility of a simple, one-stage procedure to correct the deformed tracheal wall by irradiating it with the laser through an endoscopic approach.

From the Otolaryngology Research Center for Advanced Endoscopic Applications and the Department of Otolaryngology–Head and Neck Surgery, New England Medical Center and Tufts University School of Medicine, Boston, Massachusetts. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of New England Medical Center.

Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, May 2-4, 1995.

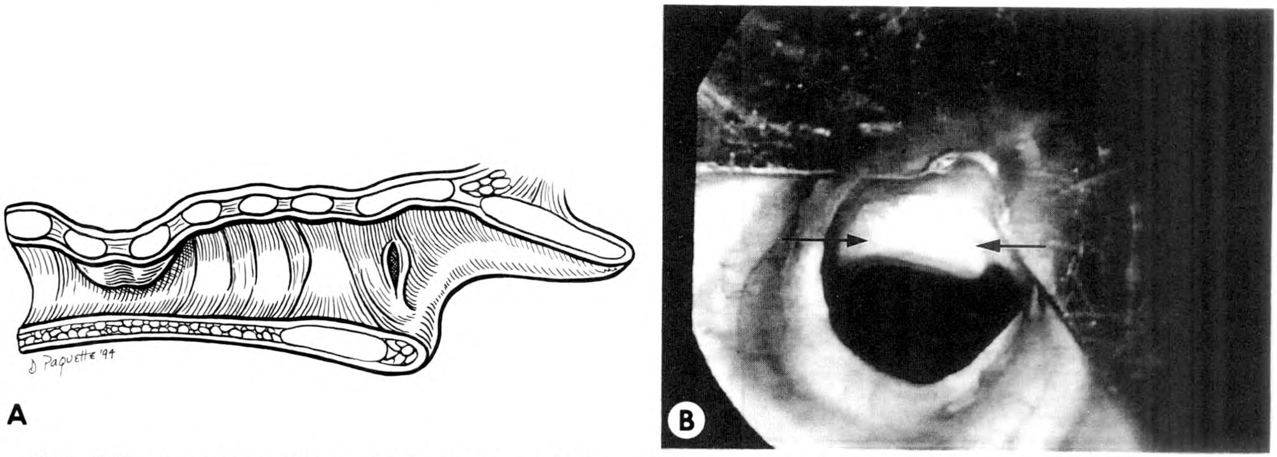

Fig 1. Collapsed tracheal wall before laser reshaping. A) Schematic diagram illustrating creation of animal tracheal wall collapse. B) Endoscopic photograph in canine model 6 weeks after creation of cartilage collapse. Arrows — collapsed tracheal wall.

MATERIALS AND METHODS

Six beagle dogs weighing 12 to 15 kg were used for the in vivo study. The experiment consisted of two phases. In phase 1, an animal model of anterior tracheal wall collapse was created and allowed to heal. Under general surgical anesthesia (pentobarbital, 15 to 20 mg/kg body weight), an H-shaped incision in the anterior tracheal wall was made through the third and fourth cartilaginous rings. The middle portion, 4 mm in width, of these rings was then excised. The two incised cartilage rings were approximated and in 6 weeks a collapsed anterior tracheal wall, 6 mm in superior-to-inferior length, was formed (Fig 1).

In phase 2, the deformed tracheal wall was corrected. Six weeks after the initial operation, under similar general anesthesia, a suspension laryngoscope, modified for exposure of the cervical trachea, was used to gain subglottic exposure. The 1.44-µm wavelength of an Nd:YAG laser (Opmilas 144, Carl Zeiss, Thornwood, NY) was used. The laser was operated in a pulsed mode (pulse width 650 microseconds) and delivered through a 600-µm core low OH quartz fiber. The animals were divided into experimental and control groups of three animals each. The animals in the experimental group had a corrective procedure with the laser reshaping technique. Endoscopically, an incision with a 1.06-µm Nd:YAG laser in contact mode at 25 W power was made around the deformed tracheal wall to release the cartilage rings. The tracheal wall was forced superiorly into a near-normal position and then irradiated with the 1.44-µm Nd:YAG laser set at 20 W power (0.1 J per pulse at 20 Hz) with an impact spot of 3 mm on the mucosal side of the cartilage surface, along the line on which maximum stress was expected. The laser beam was moved with a velocity of 1.5 to 2.0 mm/s along the line of greatest curvature on the deformed cartilage. The irradiation continued (5 to 6 minutes) until external force was no longer necessary

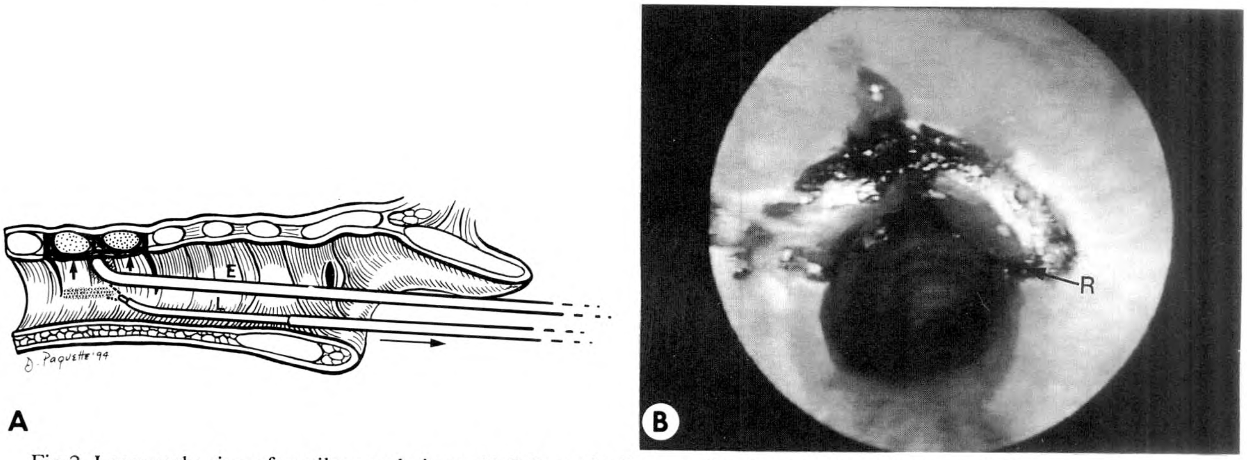

Fig 2. Laser reshaping of cartilage technique. A) Schematic diagram showing two instruments used. E (top) — "elevator" designed for endoscopic use to allow surgeon to return tracheal wall to near-normal position. L (bottom) — laser handpiece that enables laser beam to be moved along stress line of cartilage. B) Endoscopic photograph taken immediately after laser application. Note laser reshaping line (R) after application of noncontact 1.44-µm neodymium:yttrium-aluminum-garnet laser. Displaced cartilage ring is now recontoured in normal position.

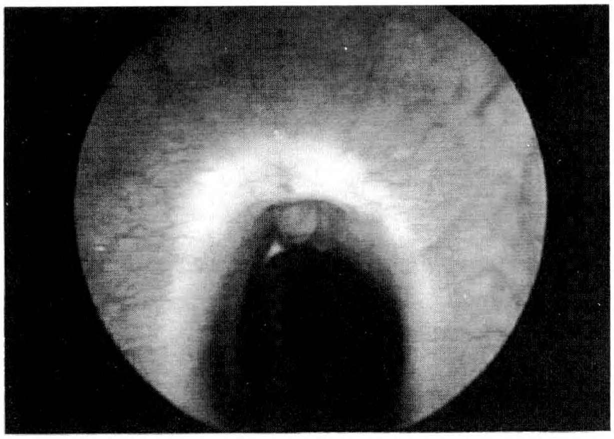

Fig 3. Adequate airway 6 weeks after laser reshaping.

to hold the tracheal rings in the correct position (Fig 2). The animals in the control group underwent a corrective procedure with stent placement. After releasing the collapsed tracheal cartilage with a laser incision, a 2.0 nylon suture was introduced through the neck skin into the airway, withdrawn through the mouth, and passed through silicone rubber tubing with a 12-mm external diameter. The stent was positioned in the airway when the suture was passed and tied externally on the neck. The stents were removed endoscopically in 4 weeks.

All animals underwent endoscopic examination at 4 and 6 weeks after the corrective operation and then were painlessly sacrificed. The tracheal specimens were excised, processed for light microscopy, and stained with hematoxylin and eosin for histopathologic examination.

RESULTS

In animals in the experimental group, after the corrective operation, the tracheal rings assumed and

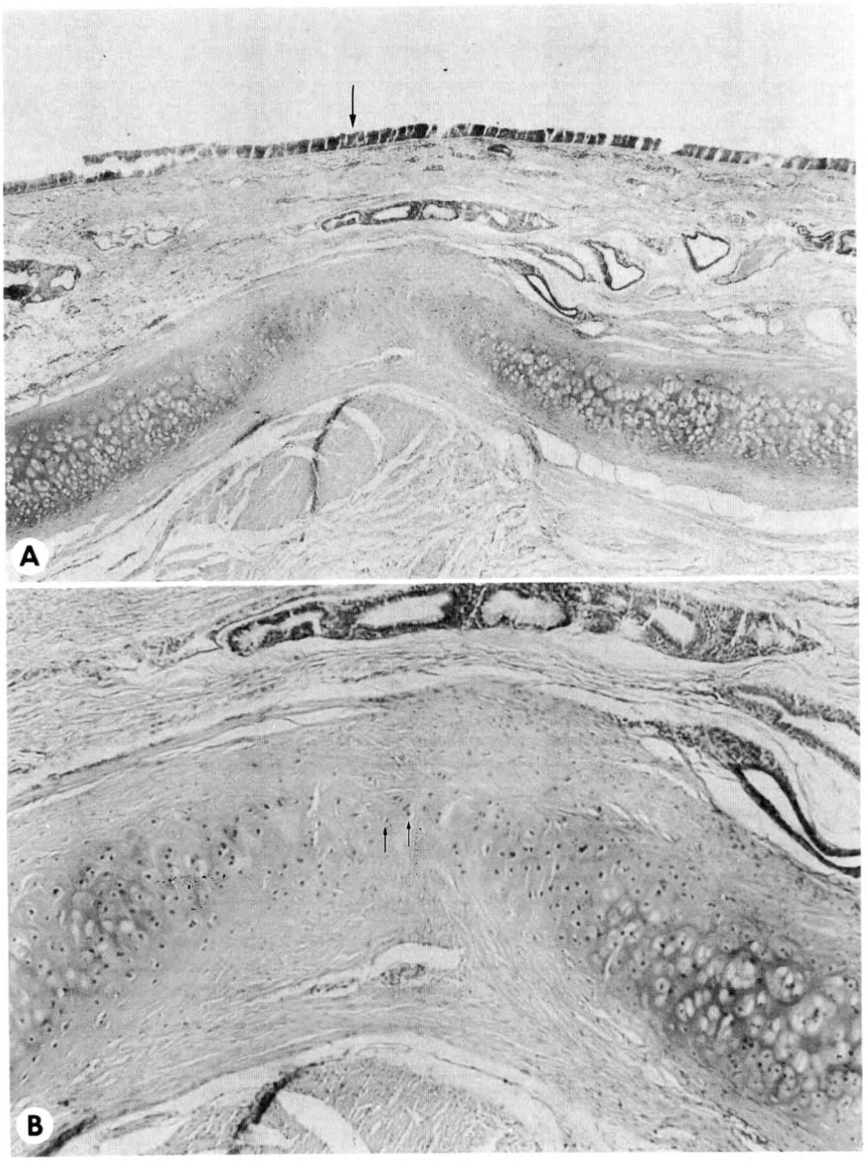

Fig 4. Histopathologic specimen of cartilage site 6 weeks after reshaping. A) Irradiated area of cartilaginous ring and overlying regenerated mucosa (arrow) with normal ciliated epithelium (H & E, original ×100). B) Numerous viable chondrocytes (arrows) in reshaping site (H & E, original ×200).

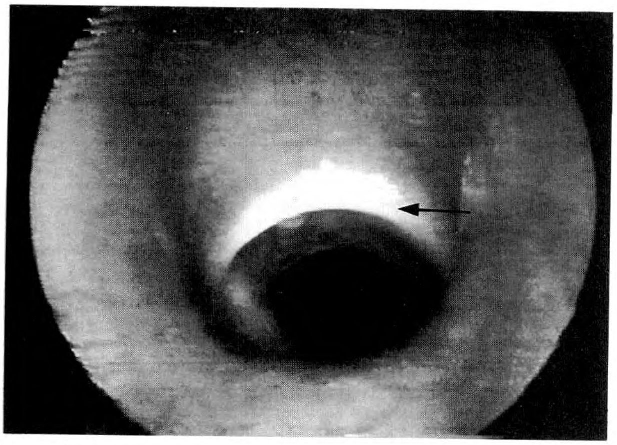

Fig 5. Endoscopic photograph from stenting control group 6 weeks postoperatively. Note that airway space was associated with mild degree of stenotic scar formation (arrow) at 6 weeks.

retained their corrected shape. Endoscopically, there was some thermal damage around the incision lines produced with the laser incisions. The mucosa at the 1.44-μm Nd:YAG laser irradiation sites was desiccated and coagulated. The airway otherwise appeared normal. After 6 weeks, endoscopic examination showed an adequate airway lined by mucosa that appeared smooth and normal, with very little granulation tissue (Fig 3). Histologically, at the site of 1.44-μm Nd:YAG laser irradiation the mucosa was fully regenerated and a number of viable, normal-appearing chondrocytes were observed in the local cartilage (Fig 4). The anterior-posterior inner tracheal diameters in these animals were 10, 9, and 10 mm, respectively. In the control group, there was also an adequate airway with intraluminal diameters of 11, 9, and 10 mm, but stenotic scarring was present at both ends of the stent, with a significant inflammatory response in the local mucosa (Fig 5).

DISCUSSION

Endoscopic laser techniques for treatment of subglottic and tracheal stenoses have been most successful when used as a minimally traumatic, mucosal-sparing approach.[8] This success, however, is limited to correction of intraluminal stenosis caused by pathologic soft tissue changes such as fibrosis with or without inflammation. An open approach, with or without stenting, is still recommended for treatment of stenosis associated with a collapsed or deformed cartilaginous wall.

That reshaping of cartilage with the laser is a simple procedure enhances its potential for clinical application. In our in vitro study, cartilage rings obtained from canine trachea could be changed to a completely new shape by the 1.44-μm Nd:YAG laser, without visible carbonization or tissue ablation. Cartilage rings were reshaped to a straight contour, or even to an inverse curvature, following application of the laser at low power, moved slowly along the lines of maximum stress.[7] In the in vivo experiments, this tendency continued. The treated cartilage retained its new shape and functional elastic strength to resist external force for up to 6 weeks — up until the date of sacrifice. The exact mechanism for laser reshaping of cartilage is not well understood, but is considered to be related to the redistribution of intracartilage water produced by the laser heating. Cartilage comprises three main elements: chondrocyte cells, extracellular matrix, and bound water. The resilience of cartilage and its ability to resume an original shape and texture after removal of a deforming force result directly from the water-structuring property. When external force is applied on cartilage, the contained water is displaced. As soon as the force is removed, water returns to the cartilage and the original shape is restored immediately. Moderate heating of cartilage induces breakage of water molecular bonds and redistribution of water, resulting in the significant increase of material plasticity.[6,9]

Chondrocytes have limited regenerative ability, and self-regeneration of cartilage contributes little to the healing of a defect. Similarly, cartilage tissue has shown very little ability to regenerate following laser surgery.[10,11] In the in vivo study, however, numerous chondrocyte cells mixed with some fibrous tissue could be found in the reshaped areas 6 weeks postoperatively. One possible explanation for the survival of chondrocytes is that the laser heating was minor to moderate and produced little thermal damage. Histologically, there are easily recognizable changes in the laser-irradiated areas, but with limited and potentially reversible damage (Fig 4). These are similar to the changes noted by Helidonis et al.[6] In their study of the acute response following laser cartilage reshaping, histologic examination using both light and electron microscopy showed two tissue layers having different morphologic changes. A very superficial layer with maximum damage was found in close vicinity to the laser-irradiated surface. This layer contained noticeably damaged chondrocytes with hardly distinguishable nuclei and shrunken cytoplasmic components. In other, deeper layers involving most of the sample's thickness, however, the cells and matrix did not show any significant differences from normal ones. Also, it was demonstrated that the stress relaxation process associated with redistribution of water, during cartilage reshaping, took place at a temperature of 70°C to 75°C, which is much less than that required for vaporization of tissue.[12] The histomorphologic results can only give information about the cells' architecture, without any indication of their functional state. Endoscopic examination showed, however, that the reshaped cartilage was functionally strong enough to support an airway lumen.

The particular laser wavelength used has a decisive effect on the depth of tissue absorption, which, in our experience, in turn is key for successful cartilage reshaping. Ideally, the heated area should be deep enough to relax the internal stress of the cartilage but still preserve chondrocytes and the outer perichondrium, the cartilage's only source of blood supply and nutrition. In this study, the 1.44-μm Nd:YAG laser is chosen as a more effective laser.

Apparent inconsistency between our results and those of earlier work by others[4-6] preferring the CO_2 laser may be due to the different types of cartilage used. The thickness of the dog's tracheal ring is 1.8 to 2.5 mm, significantly greater than the 0.4 to 1.0 mm of rabbit ear cartilage. The theoretic penetration depth in tissue for the CO_2 laser is about 0.016 mm, which may be effective for thin cartilages. The 1.44-μm wavelength with a penetration depth of approximately 0.3 mm should be better for reshaping thicker cartilage. To induce stress release in thicker cartilage, more molecular bonds must be broken. This translates into deeper penetration with adequate absorption in depth to produce minimal to moderate heating without surface carbonization.

Intraluminal stenting, with various designs and materials, is one of the most popular techniques in laryngotracheal reconstruction. The stents can be used to expand a stenosed area and provide a scaffold for the growth of epithelium. Stenting is especially valuable when using a free cartilage graft to reconstruct an airway or when supporting an unstable cartilage framework. There have been reports to demonstrate and to support its clinical use.[13] Unfortunately, stenting often produces local granulation tissue and infection despite new materials and placement techniques.[14,15] The production of granulation tissue will very often delay decannulation and induce recurrent stenosis, particularly in young patients. In our study, a tubular-shaped silicone stent was placed and secured in place endoscopically. The results show that the intraluminal stent is effective in maintaining an adequate airway space, but the local tissue reaction remains severe despite the minimal trauma associated with placement.

The results of our study show that it is possible to reshape deformed tracheal cartilage without vaporization or carbonization by using the 1.44-μm Nd: YAG laser endoscopically at low power. The treated cartilage retains its new shape and elastic force to sustain a functional airway.

Before this technique can be clinically used, some technical improvements are necessary. It was difficult to make an accurate endoscopic incision to release the collapsed cartilage. A new handpiece with an appropriate or even adjustable firing angle is needed to make a full-thickness cartilage incision and spare the subcutaneous tissue. Additionally, a special instrument is needed to force a freed and displaced cartilage into correct position and maintain it while laser reshaping irradiation is in progress. These technical problems can be easily addressed after further animal laboratory research with long-term survival of cartilage and a better understanding of the basic mechanism of laser-cartilage interaction.

To focus our study on treatment of the deformed cartilaginous ring rather than soft tissue scar formation, we tried to create a "pure" cartilage collapse by making an H-shaped incision in the anterior wall, which is a major site of tracheal buckling following a tracheotomy.[16] The cut cartilaginous rings were pushed posteriorly and then fixed in a collapsed position. To the best of our clinical judgment, through endoscopic examination at 6 postoperative weeks, this area of narrowing represents cartilage primarily, with perhaps minor soft tissue scar formation (Fig 1B). It appears that this cartilage reshaping technique, at least in its present form, is applicable to selected cases with "pure" posterior displacement or a buckling type of deformity of the central-anterior aspect of the trachea. Patients with severe malacia or absence of cartilage, which is common in patients with tracheal collapse, are not candidates for this procedure.

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LARYNGEAL AND TRACHEAL RECONSTRUCTION

The Second International Symposium on Laryngeal and Tracheal Reconstruction will be held in Monte Carlo, Monaco, May 22-26, 1996. For more information, contact Tihana Barisic, Publi Creations, 44, Boulevard d'Italie, Monte-Carlo, MC, 98000 Monaco; telephone +33-93-159976; fax +33-93-159977.

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EPITHELIAL HYPERPLASTIC LESIONS OF THE LARYNX

The International Conference on Epithelial Hyperplastic Lesions of the Larynx will be held October 28-30, 1996, in Ljubljana, Slovenia. For more information, contact Bostjan Luzar, MD, or Aleksandar Vodovnik, MD, Institute of Pathology, Korytkova 2, 61105 Ljubljana, Slovenia; telephone 386 61 1403042 (Int. 268); fax 386 61 301 816; E-mail Vodovnik@IBMI.MF.UNI-LJ.SI.

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VOCAL FOLD ATROPHY AND ITS SURGICAL TREATMENT

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The effectiveness of surgical treatment for vocal fold atrophy of various causes was examined. Type I thyroplasty was performed on 31 patients with vocal fold atrophy, often bilaterally and occasionally combined with type III thyroplasty. Of these 31 patients, 3 patients underwent the operation twice, for a total of 34 operations. The surgery was found to be effective in improving the voice quality and the ease of phonation. When a scar or sulcus was present, the results were not as satisfactory. The intraoperative decision-making process as to which procedure to perform is extremely important for achieving an optimal voice, and should be based on the intraoperative voice quality, fiberoptic findings, and manual tests. In operations for vocal fold atrophy in which the vocal folds are mobile, a silicone shim should be firmly fixed to prevent migration. Overcorrection is generally recommended. No complications were encountered. It was often the increased ease of phonation rather than the improved voice quality that patients appreciated after surgery.

KEY WORDS — aging voice, laryngeal framework surgery, phonosurgery, thyroplasty, vocal fold atrophy.

INTRODUCTION

Vocal fold atrophy, with or without sulcus vocalis, has not attracted much clinical attention. This is probably because the disease has been generally regarded as being incurable. However, various treatments have been recently attempted with various degrees of success. These strategies included voice training, intracordal injection,[1,2] and thyroplasty.[3] Summary reports on this pathologic entity[4-11] seem to be increasing, reflecting a growing interest in the disease. The expected gerontologic problems of the world's aging population[12-14] also support the increasing importance of treating this disease. This report analyzes the results of surgical treatments for vocal fold atrophy, with special reference as to whether there is any difference in its effectiveness depending on the cause of atrophy or laryngeal findings. Differences in surgical technique for vocal fold paralysis versus atrophy are also stressed.

SUBJECTS AND SYMPTOMS

The subjects consisted of 31 patients who had undergone one or two laryngeal framework operations as treatment for vocal fold atrophy over the last 23 years. The probable etiologic factors were multiple. Cases of vocal fold atrophy can be divided into seven etiologic groups. The "vocal abuse" group consisted of 3 salesmen, 3 cram school teachers, and 3 priests, who use their voices professionally. One of the priests had undergone a prior cordal injection with silicone at another institution. The second group, with vocal fold atrophy due to apparent aging (6 patients), seems to be increasing in frequency. The third group had atrophy as a result of trauma or scarring: 2 cases of laryngeal trauma, 1 case of repeated operations for laryngeal papilloma, and 1 case of trauma, presumably due to a prolonged intubation period for cardiac surgery. Excessively high vocal pitch in young males, such as the typical "mutational voice disorder," is occasionally associated with vocal fold atrophy. In cases in which this could not be corrected by 3 months of voice therapy, surgery was performed. This group (4 patients) was called the "mutation-related?" group because its causative relation was not always definitive. The other groups were those with familial occurrence (4 patients), hormonal disease (1 patient), and nonspecific causes (3 patients).

Common symptoms of vocal fold atrophy include a breathy and weak voice, an excessively high vocal pitch, and difficulty or fatigability during phonation.

EXAMINATION

Since the operations were performed over a long period of time at several hospitals, the follow-ups of the patients were not always available, and the examinations were not consistent. However, a minimum of at least one preoperative and one postoperative video recording was obtained for all of the patients.

Voice Recording, Subjective Judgment, and Acoustic Analysis. Simultaneous recordings were made of the fiberoptic laryngeal findings and the voice on a videotape before and after the operation. The voice

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AC / DC ratio (glottal flow efficiency)

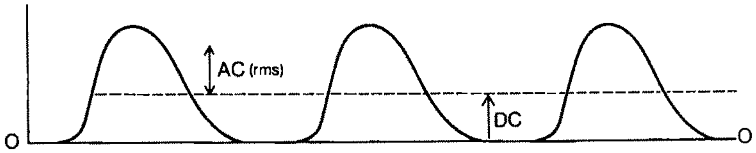
AC : alternating current = sound component of the glottal air flow

DC = direct current (mean flow rate)
of the glottal air flow

Effective value of AC = $\sqrt{\frac{\sum_{i=1}^{n} V_i^2}{n}}$, root mean square (r. m. s)

AC / DC = rms of AC / mean flow rate

Threshold value of AC / DC ≑ 50 (0.5)



Glottal flow efficiency is defined as the ratio of *AC* to *DC* of the glottal air flow

Fig 1. Glottal efficiency index as expressed by ratio AC/DC serves to assess glottal function to transform exhalatory airflow into pulsed flow or sound. AC — alternating current components of flow (ie, sound); DC — direct current components of flow or mean flow rate.

quality was then judged subjectively[3,15] on a 4-point scale (0 = normal, 1 = slight, 2 = moderate, 3 = extreme) in terms of degree of hoarseness, roughness, and breathiness. Finally, the voice was submitted for acoustic analysis with the use of a sonagraph. The outcome criteria used to express the overall results of the surgery were “almost normal” (a rating of less than 1.0) and “slightly hoarse” (a rating between 1.0 and 2.0). The voice quality was assessed during conversational speech at a habitual pitch.

Glottal Efficiency Ratio. The ability of the glottis to modulate exhalatory airflow (direct current [DC]) into a train of air puffs or sound (alternating current [AC]) was assessed with the AC/DC ratio[3,16] (Fig 1), which was measured with a phonation analyzer. This index represents how efficiently the exhalatory airflow is transformed into sound at the glottis. The details of this technique have been described elsewhere.[16]

Manual Compression Test. The manual compression test, ie, medial compression of the thyroid alae with the fingers at the level of the glottis, is an essential test in deciding on the indications for medialization surgery and predicting the outcome.[3,17] If the vocal folds are covered with extensive scarring, if the glottal chink due to atrophy is rather wide, or if the thyroid alae are calcified, then the effects of manual external medialization of the vocal folds on the voice would not be as great as expected.

The manual compression effect on the voice in vocal fold atrophy is usually less pronounced than in unilateral vocal fold paralysis. If the compression does produce a significant improvement in the voice quality, then postoperative improvement in the voice can be assured to the patient, at least to an extent equivalent to that obtained by the manual compression, and perhaps more. Manual medial compression, combined with anteroposterior compression for vocal fold relaxation or with cricothyroid approximation for vocal fold tensing, is used as an adjunct test to determine the type of surgery to be performed. However, the final decision as to whether a type III thyroplasty or a cricothyroid approximation is needed is made by intraoperative tests.

TABLE 1. TYPE OF THYROPLASTY EMPLOYED

Type	*No. of Operations*
I (unilat)	9
I (bilat)	6
I + III (unilat)	10
I + III (bilat)	3
I (second)	5
III (second)	1
Total	34

SURGICAL TECHNIQUE

Selection of Technique. The surgical options available included 1) unilateral or bilateral thyroplasty type I and 2) type I combined with thyroplasty type III or with cricothyroid approximation.[2] The decision is usually made intraoperatively. However, the side that is operated on first is decided preoperatively, according to which of the two vocal folds appears to be more atrophic. Table 1 shows the types of surgery employed in our group of patients.

Special Techniques for Vocal Fold Atrophy. Cor-

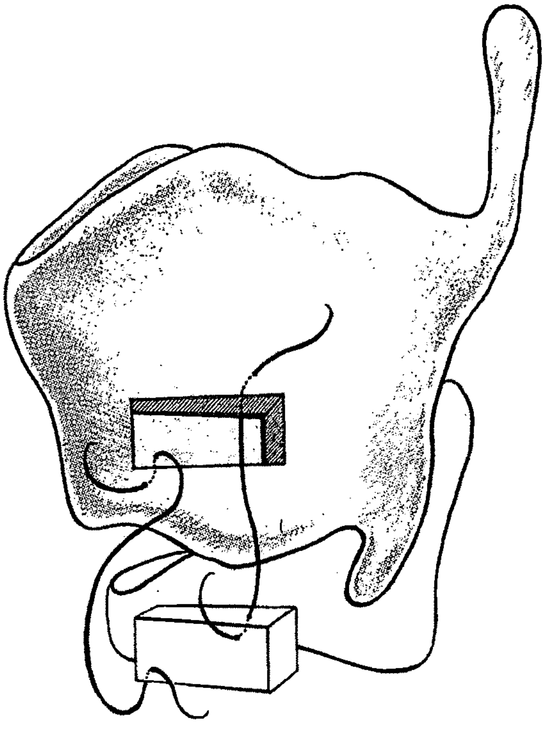


Fig 2. Type I thyroplasty. Salient points in surgical techniques for vocal fold atrophy include more medialization, firm fixation of shim, and bilateral procedures as needed.

rection of the concavity of the vocal fold is achieved by thyroplasty type I, in essentially the same manner as for a paralyzed vocal fold. Under local anesthesia, the thyroid ala is exposed to create a window at the level of the vocal fold. Although the detailed surgical technique has been described in previous reports,[3,18,19] for vocal fold atrophy the rectangular cartilage piece should frequently be pressed more deeply than for vocal fold paralysis in order to enhance the medialization effect. Subchondral undermining around the rectangular window should be performed sufficiently for the window to shift medially without much resistance, but not too far from the window edge line (approximately 2 mm in width). Fixation of the window cartilage and silicone shim should be done firmly to prevent them from migrating (Fig 2), because they are more likely to migrate with the mobile vocal folds. Depending on the voice and the fiberoptic laryngeal findings, a type I thyroplasty can be added on the contralateral side as needed.

Additional Surgery for Changing Vocal Fold Tension. Before concluding the surgery, the vocal folds are tested for the effects of their tensing or relaxation on the voice quality. For relaxation, the thyroid cartilage is pressed posteriorly during phonation at the point at which the anterior commissure projects. For tensing, an elevator is set under the inferior edge of the cricoid and is lifted toward the thyroid cartilage during phonation. The type III thyroplasty was often modified to achieve relaxation as well as medialization of the vocal fold, as shown in Fig 3.

RESULTS

General Results. Figure 4 shows an example of the surgical results. The overall success rates of the surgery are shown in Table 2. The poor results obtained in 5 cases were all from patients who were operated on during the initial period of instituting surgical treatment for atrophic vocal folds decades ago. During this early period, only unilateral thyroplasties were performed, probably insufficiently in some cases, and with excessive caution against postoperative complications such as dyspnea. Furthermore, 3 of the 5 patients had wide or moderate scarring in addition to the atrophy. Two of the 3 patients underwent a second operation after mucosa grafting, and were finally classified into the "slightly hoarse" group. One of these 5 patients had undergone a prior cordal injection with silicone, and even two thyroplasties failed to improve the voice quality to his satisfaction.

The overall results were apparently inferior to those obtained for vocal fold paralysis in terms of voice quality. The ease of phonation, however, significantly improved in the majority of cases, both subjectively and with an increment of the AC/DC ratio over 10. No complications occurred in any of the cases.

Differences in Results Depending on Cause of Atrophy and Laryngeal Findings. An analysis of the surgical results shows that in the almost normal group, only 2 of 15 patients (13%) had a sulcus,

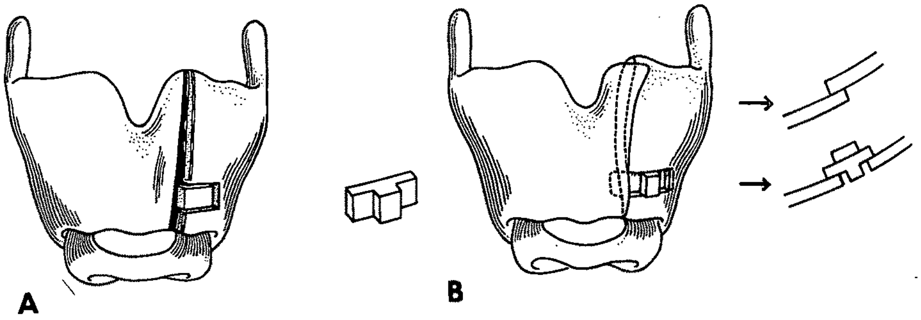


Fig 3. Combination of type I thyroplasty and type III thyroplasty for medialization as well as relaxation of vocal fold. A) Cartilage incision. B) After fixation.

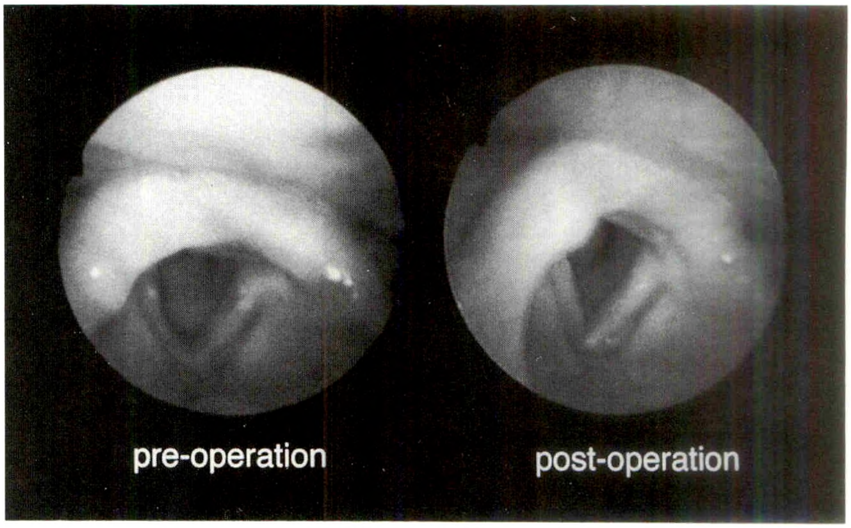


Fig 4. Laryngeal pictures of right atrophic vocal fold, before and after operation.

whereas in the slightly hoarse group, 9 of the 12 patients (75%) had a sulcus. Apparently, atrophy with a sulcus has a much poorer prognosis. However, it should be noted that sulcus vocalis is not uniform; some cases were identifiable only on abduction, and other cases were visible regardless of the position of the vocal fold. The former type was more likely to be seen in less severe cases of vocal fold atrophy, and this was the type of sulcus in which an almost normal voice could be obtained by surgery.

It is difficult to draw any general conclusions on which method is more suitable for treating vocal fold atrophy from the present data, because the type of surgery was selected on the basis of intraoperative test results. However, except for cases of slight atrophy, we tended to use bilateral type I thyroplasty or combined thyroplasty with type III with increasing frequency.[19]

DISCUSSION

Terminology. Terminology is often confusing in laryngology: vocal cord versus vocal fold, bowing versus atrophy of the vocal fold, and sulcus versus corrugation or wrinkle of the vocal fold. We feel that the use of the term "cord" versus "fold" does not matter very much, but it would be convenient to standardize them. However, if "cord" is discarded, then the terms cordal injection and cordectomy would have to be revised.

TABLE 2. RESULTS

	*No. of Evaluations**
Voice	
Improved to almost normal	15
Improved but slightly hoarse	12
No remarkable change	5
Ease of phonation	
Much improved	19
Improved	9
No remarkable change	4

*One patient underwent two thyroplasties and mucosa grafting was evaluated twice, for a total of 32 evaluations.

Those who are against the use of "atrophy" may claim that without histologic or electromyographic examination, the diagnosis of "atrophy" cannot be supported. However, in our daily practice, the vocal fold is often described as "hypertrophic" on the basis of a visual impression. The use of "vocal fold atrophy" in this article means nothing more than the antonym of hypertrophy or simply a reduction in volume. It does not necessarily imply atrophy of the vocalis muscle due to denervation or degeneration, but rather implies atrophy of the muscle, the mucosa, or any structure in the vocal fold.

"Bowing" gives a somewhat two-dimensional impression and does not necessarily match our three-dimensional clinical impressions obtained by viewing the folds at various phases of respiratory and phonatory movements. The term "bowing" may also lead to the erroneous concept that it is due to excessive relaxation of the vocal fold. In fact, paralysis of the cricothyroid muscle alone does not produce bowing of the vocal fold. Electromyographic examination does not seem capable of revealing the cause of vocal fold atrophy, because it provides information only from the very limited area of the vocal fold in which the electrode tip is placed, and one can only assume that it was placed in the right location. Furthermore, since the type I thyroplasty was designed for the medialization and augmentation of the vocal fold and not the restoration of neurologic function, it does not matter what the cause is. The same results are achieved regardless of whether the folds are denervated, degenerated, atrophied secondary to inflammation or ischemia, hormonally compromised, or senile. What matters is the extent of atrophy and

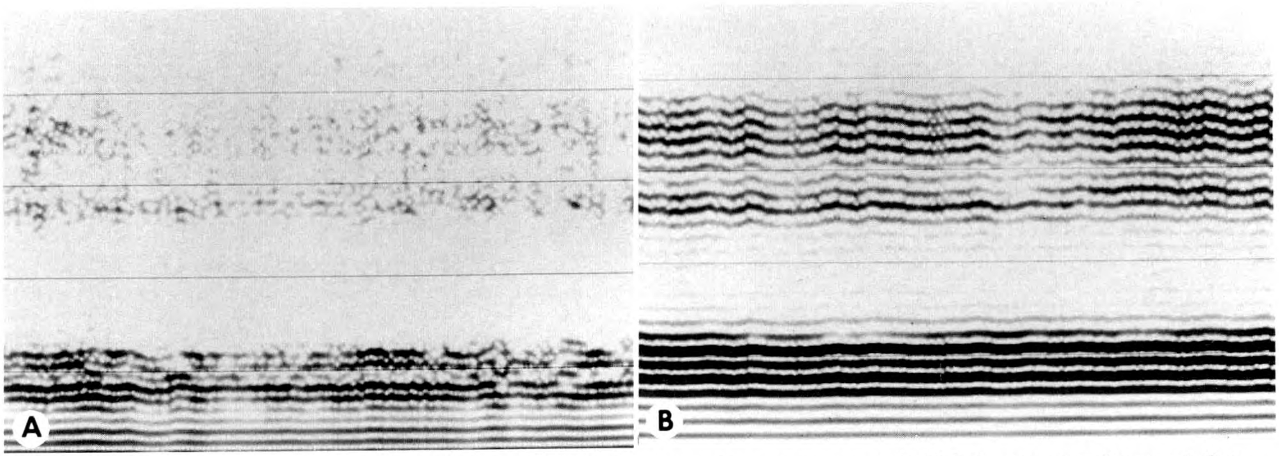


Fig 5. Sonagrams for vocal fold atrophy A) before and B) after operation. Noise components in high-frequency region seen before operation were entirely replaced by harmonic components after operation.

the rheology of the vocal fold. Denervation atrophy of the vocal fold secondary to paralysis is explicitly excluded from the present study. Another problem in semantics is the use of "sulcus" and its variations. Some sulci are recognizable regardless of the position of the vocal fold, but others emerge only on abduction or as a result of subglottic edema. These terms should be differentiated from each other, especially when surgical indications have to be considered.

Indication and Selection of Surgery. On the basis of our results that there was neither deterioration nor complication in any of the cases, a more proactive stance seems justified for treating vocal fold atrophy surgically. Type I thyroplasty, whether combined with type III or not, is generally the primary indication for vocal fold atrophy. However, the prognosis is different, depending on other laryngeal findings. The surgical results clearly indicate that those cases with a sulcus or scar are more difficult to treat surgically.[19] Vocal fold atrophy complicated with widespread scarring may be better treated with mucosal grafting first, and then followed by type I thyroplasty as needed.[20] Familial vocal fold atrophy is often accompanied by a sulcus, and also presents a difficult problem for surgery.

With respect to age-related atrophy, there is the concept that muscle atrophy is an inevitable aging phenomenon, and cannot be corrected by surgery. This certainly holds true, but the shape or volume of the atrophic vocal fold is more critical for glottal function than diminished contraction force of the vocalis muscle, and altered vocal fold shape can certainly be compensated for by surgery to some extent. Vocal fatigue and a weak voice due to vocal fold atrophy, and the consequent air loss on phonation, seem to deprive aged but young-in-spirit people of much energy and confidence. This surgery can greatly improve the quality of life in these people. Vocal training may be indicated if the glottal chink on phonation is narrow, but in most of the severe cases, surgery would be more efficient as well as less burdensome to the patient. It may also facilitate postoperative voice training when needed, by a pump-priming effect. The decision to add a type III or IV thyroplasty is determined by intraoperative tests to change vocal fold tension. When the preoperative vocal pitch is very high, a type III thyroplasty for relaxing the fold may be more indicated, though. Vocal fold tensing techniques, whether combined with a type I thyroplasty or not, have been used successfully by some surgeons instead of relaxation, for glottal insufficiency[21-23] and sulcus vocalis.[24] A supplementary medialization effect may also be expected following cricothyroid approximation, as previously reported.[25] It seems unlikely to us that the cricothyroid approximation technique can straighten a bowed or lax fold, because it is unlikely that bowing is due to defective function of the cricothyroid muscle.[3] In fact, Tanaka et al[10] reported that cricothyroid muscle activity had no significant relationship to vocal fold bowing. The issue as to whether the vocal fold should be stretched or relaxed remains controversial. We subscribe to the policy that "good results come first, theory comes next."

Evaluation. It is of great interest to note that better surgical results were obtained for ease of phonation rather than for voice quality. Furthermore, it often was this ease of phonation that patients, especially patients in the vocal abuse group, appreciated the most, as speaking in a loud voice to a large audience is part of the lifestyle of these patients. Ease of phonation is not easy to assess. Measurements of the mean flow rate during phonation or the maximum phonation time may be of some help, but theoretically, the glottal efficiency index or the AC/DC ratio would be more appropriate, because it also takes into account the intensity of the voice signal. The theoretic details have been described elsewhere,[3,16] and the clinical usefulness of this index has been reported by Kitajima[26] and Kitajima and Fujita.[27] However, it is emphasized here that these measurements should be made several times at a consistent point during the phonation, preferably in the middle of it. Thus, we ought to direct more attention to this aspect of phonation, and an objective method for assessing ease of phonation remains to be developed. A cram school teacher is illustrated with sonagrams (Fig 5) to represent those patients who showed marked improvement in both quality of voice and ease of phonation.

Surgical Technique for Vocal Fold Atrophy. More medialization may be required for vocal fold atrophy correction than for paralysis. Our experience indicates that this can be accomplished without fear of causing dyspnea, and is frequently performed with the use of bilateral procedures. The cartilage piece at the bottom of the window should be sufficiently freed from the window frame in order to achieve greater medialization. This can be performed by careful undermining between the cartilage frame and the perichondrium by about 2 mm in width. In cases of mobile vocal folds, a greater possibility of shim migration may be expected, and therefore preventive measures such as retaining the bottom cartilage in place and firm fixation of the shim with sutures are recommended. Further variations may be possible in combining type I thyroplasty with type III.

CONCLUSIONS

Unilateral or bilateral type I thyroplasties, and those combined with type III, have been performed in 34 cases of vocal fold atrophy over the past 23 years. Though not as good as the outcome for vocal fold paralysis, these results seem promising and are especially good for patients without sulci. Vocal fold atrophy with widespread scarring is better treated with mucosal grafting first, followed by type I thyroplasty.

The surgical technique differs from that for vocal fold paralysis, and includes more medialization, firmer fixation of the shim, and the possibility of combination with tension-altering techniques. In assessing the surgical outcome, the quality of the voice as well as the ease of phonation should be taken into consideration. Neither complications nor deterioration of the voice quality occurred in any of the cases. Surgical treatment may be recommended with more confidence than before, because it is relatively safe as well as effective for most cases of vocal fold atrophy.

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CRANIOFACIAL/CLEFT LIP AND PALATE SOCIETY OF GREAT BRITAIN

The next meeting of the Craniofacial/Cleft Lip and Palate Society of Great Britain will be held April 24-26, 1996, in Eghan, Surrey, England. For further information, contact Dr Debbie Sell, Speech and Language Therapy Department, The Hospital for Sick Children, Great Ormond Street, London, WC1N 3JH, United Kingdom; telephone (UK) -71 405 9200 ext. 5043.

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FUNCTIONAL ENDOSCOPIC SINUS SURGERY: BASIC AND ADVANCED TECHNIQUES

The course Functional Endoscopic Sinus Surgery: Basic and Advanced Techniques will be held in Graz, Austria. The English course will be held July 11-13, 1996, and the German course September 5-7, 1996. For further information, contact Prof H. Stammberger, MD, ENT Department, University Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; fax ++43 316 385 3425.

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SULCUS VOCALIS: A RATIONAL ANALYTICAL APPROACH TO DIAGNOSIS AND MANAGEMENT

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The term sulcus vocalis has been applied to a spectrum of disorders ranging from minor vocal fold indentations to destructive lesions causing severe dysphonia. To clarify the pathophysiology and to develop a more rational approach to treatment, we report a series of sulcus patients including 20 surgical cases. Clinical and histopathologic analysis produced a clinically useful classification: type 1 is a physiologic variant accentuated by atrophy but with intact lamina propria; types 2 (sulcus vergeture) and 3 (sulcus vocalis) are characterized by severe dysphonia, loss of vibratory activity, and destruction of the functional superficial lamina propria. These latter cases respond favorably to microsurgery designed to remove destroyed tissue, release scar contracture, and promote mucosal redraping by regional undermining. Further study of the extracellular matrix of the superficial lamina propria (Reinke's space) might indicate a common pathway in the pathogenesis of sulcus deformities and other related benign vocal fold lesions.

KEY WORDS — larynx, pathology, sulcus vocalis, vocal fold, voice.

INTRODUCTION

Progress in assessment and successful management of sulcus vocalis has been hampered by inconsistent terminology and a lack of agreement on the basic pathology. Sulcus is a generic anatomic term referring to an indentation, groove, or furrow. The earliest mention of sulcus vocalis was probably in 1892 by the anatomist Giacomini,[1] who described a sulcus deformity of the vocal fold. Bouchayer and Cornut[2] distinguished a focal invagination of epithelium attaching to the vocal ligament (sulcus vocalis) from sulcus vergeture, which they described as a linear depression along the medial margin of the vocal fold. Lindestad and Hertegård[3] chose not to distinguish these, lumping them in the "sulcus" group; both groups exhibited spindle-shaped glottal insufficiency. Since the clinical appearance of many forms of spindle-shaped glottal insufficiency can be similar, patients with sulcus vocalis, furrows and bowing, presbylaryngis, paralysis, and connective tissue and/or thyroarytenoid muscle atrophy are often considered together. Adhering to rigorous anatomic criteria, Nakayama et al[4] identified shallow sulci in 4 of 20 larynges incidentally removed from random autopsy specimens.

There is agreement on the general clinical presentation of sulcus vocalis. Hirano and Kakita,[5] Lindestad et al,[6] and Bouchayer and Cornut[7] have described the presenting symptoms of hoarseness, vocal fatigue, asthenic voice quality, and effortful voice projection. In a recent comprehensive functional assessment of 126 sulcus vocalis patients, Hirano et al[8] described perceptual ratings, acoustic and aerodynamic functions, and analysis of videostroboscopy in this population. Most authors agree that laryngoscopic findings include spindle-shaped bowed vocal folds that fail to approximate, medial furrows seen during inhalation, impaired vibration, and frequent supraventricular hyperfunction.

Discussions on pathogenesis and treatment are less consistent and reflect a lack of agreement on terminology. Arnold[9] reviewed comparative anatomic studies supporting a congenital origin for sulci. Bouchayer et al[10] proposed that sulcus vocalis is congenital, a result of faulty development of the fourth and sixth branchial arches, and probably resulting from ruptured epidermoid cysts. They documented onset of dysphonia during childhood in 55% of sulcus patients, frequent associated cysts, failure of lesions to return after adequate excision, and descriptions of familial occurrence. Evidence for an acquired causation dates back to 1928, when Van Caneghem[11] noted onset of sulcus vocalis in adult patients with potentially provocative traumatic or infectious factors (such as tuberculosis) evident. Also, the recent review of whole-mount laryngeal cancer specimens from the University of Wisconsin[4] identified adjacent sulcus deformities in 48% of specimens, supporting argu-

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TABLE 1. DEMOGRAPHIC SUMMARY AND POSSIBLE ETIOLOGIC FACTORS IN PATHOLOGIC SULCUS GROUP

					Possible Causes								
Operative Patient	*Age*	*Sex*	*Chief Complaint*	*Onset (Years Ago)*	*Infection and/or Laryngitis*	*Vocal Abuse*	*Vocal Fold Surgery*	*Intubation*	*GERD*	*Smoking*	*Daily Alcohol*	*Family History*	*Other Disease or Allergy*
1	10	M	Hoarseness	3		+						+	
2	26	M	Hoarseness	22	+		+	+	+				
3	27	M	Hoarseness	12				+			+		
4	31	M	Hoarseness	20		+							+
5	32	M	Hoarseness	6		+			+			+	+
6	33	M	Chronic hoarseness	>20									+
7	36	M	Hoarseness	>30		+			+	+	+	+	
8	38	M	Abnormal voice	>30	+								
9	54	M	Breathy dysphonia	4				+		+		+	
10	13	F	Abnormal voice	13									
11	21	F	Hoarseness	1	+								
12	23	F	Recurrent raspy voice	5	+		+	+			+		
13	23	F	Hoarseness	3	+	+				+			+
14	27	F	Hoarseness	3	+	+	+	+	+				+
15	29	F	Recurrent hoarseness	11		+	+	+					+
16	32	F	Hoarseness, raspy voice	3	+	+	+	+	+				
17	35	F	Hoarseness	3		+							
18	39	F	Voice loss	3	+								+
19	43	F	Recurrent hoarseness	1		+	+	+					+
20	68	F	Hoarseness	>20		+	+	+			+		+

GERD — gastroesophageal reflux disease.

ments for an acquired pathogenesis.

Numerous reports of treatment results have failed to agree on suitable management strategies. This is partly due to lack of consensus about the definition of sulcus vocalis and a tendency to address only one component of the disorder. Voice therapy as a single modality has had limited success in the management of sulcus vocalis, but it is considered an essential adjunctive measure.[12] Injections of bioimplants,[13,14] Teflon,[15] and steroids,[7] as well as medialization thyroplasties,[16] have been helpful, but are inadequate without additional measures. Incision with undermining has been done in an attempt to reconstitute a looser subepithelial plane,[7] and this has been combined with vertical incisions (slicing) to break up linear tension lines.[17] Direct excision by microdissection[7] and with laser ablation (M. Remacle, personal communication and review of videotapes, 1989) have been used successfully in some sulcus vocalis cases.

In clinical practice we have observed many patients with vocal fold configurations that resemble sulcus deformities. The spectrum of disease ranges from physiologic vibratory contours revealing medial vocal fold furrows to vocal fold destructive lesions causing severe dysphonia. In an effort to clarify the pathophysiologic findings and develop a more rational approach to treatment, we focus here on a review of 20 patients with surgically treated pathologic sulcus lesions. Clinical, surgical, and histopathologic observations are described, and these lesions are contrasted to those in other patients exhibiting physiologic sulcus configurations. In describing the clinical features and histopathology, we explore common denominators that might be useful as a guide in treatment selection.

MATERIALS AND METHODS

Observation of a sulcus configuration to the vocal fold is evident in many patients evaluated in our voice clinic. To compare physiologic and pathologic cases of sulcus, we looked at two groups of subjects. We

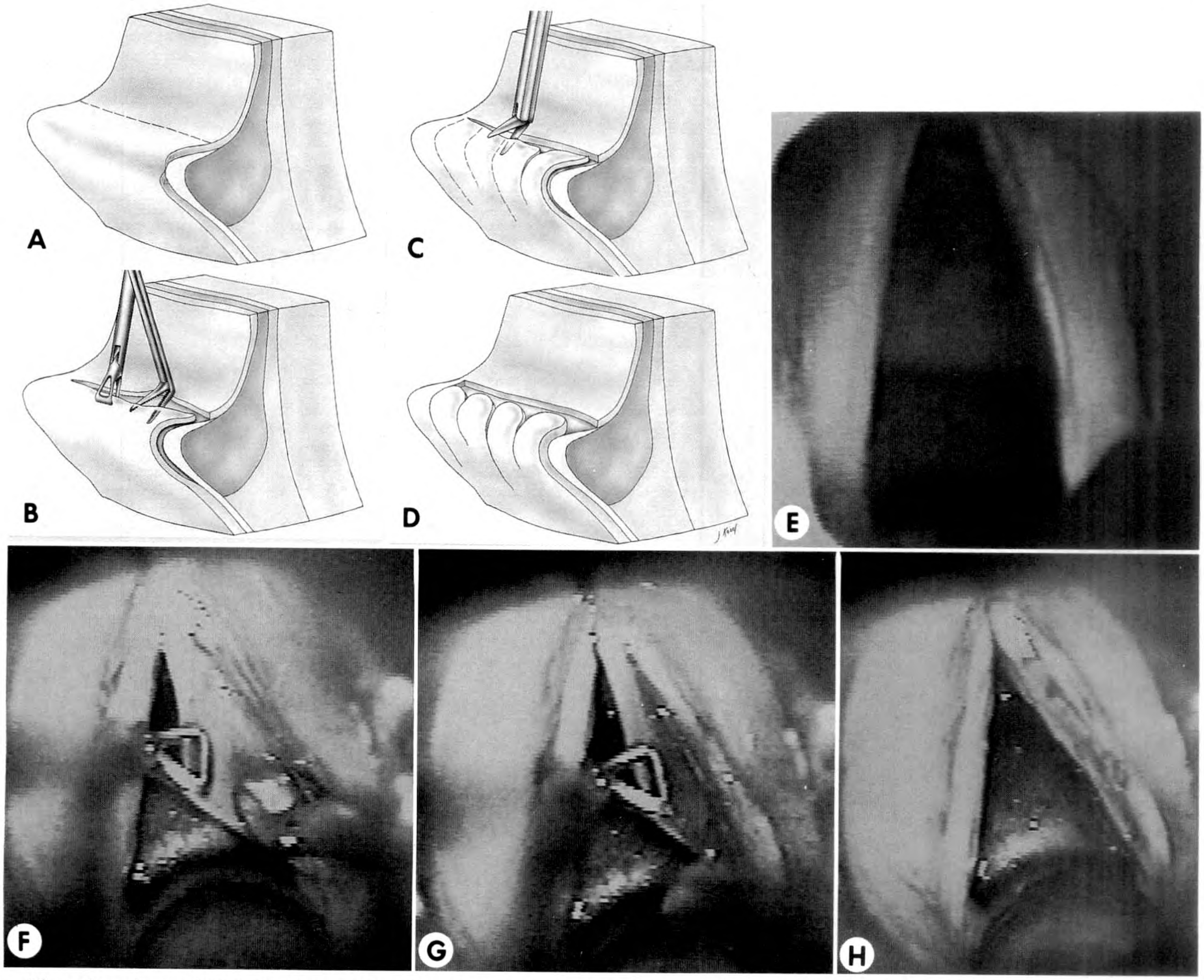

Fig 1. Steps in surgical management of type 2 sulcus (vergeture) based on technique of Pontes and Behlau.[17] A-D) Drawings. Each diagram is simplified three-dimensional medial-sagittal construct with vocal ligament in white. E-H) Operative video prints demonstrating each of same steps. A,E) Superior vocal fold incision. B,F) Separating epithelial layer from intermediate and deep lamina propria attachments. C,G) "Slicing" inferior flap that has been undermined 2 mm inferior to sulcus into segments of variable length. D,H) Rough appearance of mucosa after relaxation of contracture.

examined 116 volunteers who had no complaints, but who responded to an invitation to have their voice evaluated at no charge. All 116 presented with normal to mildly impaired vibratory activity of the vocal fold, and none exhibited focal stiffness. Nine of these subjects revealed a definite medial concavity or sulcus configuration during some phase of vibration or abduction. These 9 subjects were considered to have *physiologic* sulcus. There was nothing to suggest underlying histopathologic change in the lamina propria, and these cases could clearly be distinguished on the basis of clinical examination and videostroboscopy. All 116 patients served as controls, and underwent no treatment.

The focus of this study was 20 patients with sulcus deformity and marked vocal fold stiffness indicating loss of a functional superficial lamina propria (Table 1). The vocal fold disorder in this *pathologic* sulcus group was addressed by surgical intervention. The patients were 9 males and 11 females, ages 10 to 68 years (average 32.0, SD 13.1). The most common presenting complaint was prolonged hoarseness, with age at onset equally divided between childhood and adulthood. Each case was reviewed to identify possible environmental, focal, or systemic medical factors that might have caused the condition. Most patients had a history of laryngitis or vocal abuse; allergy and prior intubation were also common; and 25% of patients had a history of gastroesophageal reflux. Alcohol intake and smoking were not particularly common in this group. Two patients had connective tissue diseases (arthritis and systemic lupus erythematosus). A family history of chronic hoarseness was present in 4 cases.

All patients underwent preliminary voice evaluations including videostroboscopy and aerodynamic

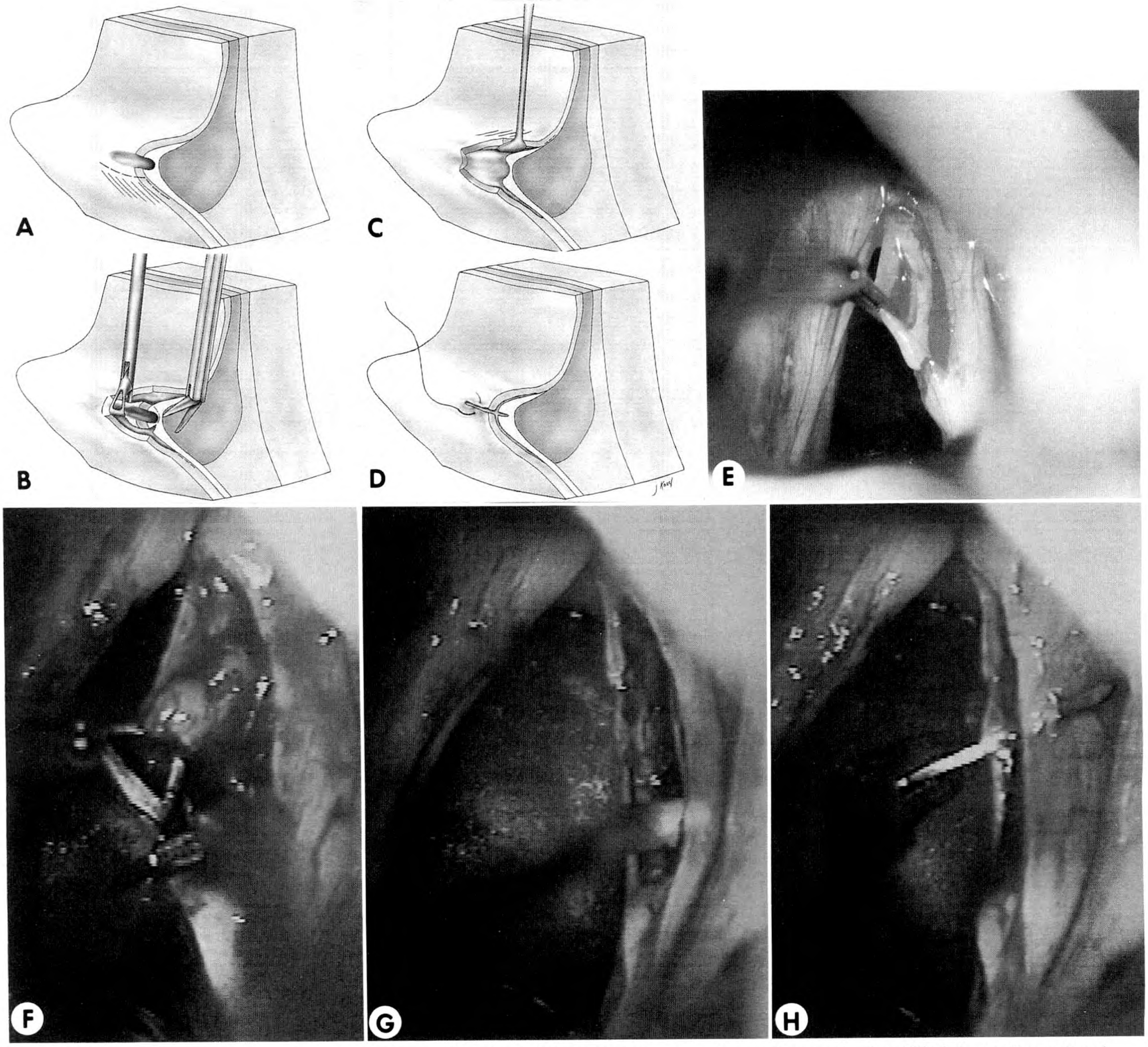

Fig 2. Steps in surgical management of type 3 sulcus (vocalis). A-D) Drawings. Each diagram is simplified three-dimensional medial-sagittal construct with vocal ligament in white. E-H) Operative video prints demonstrating each of same steps. A,E) Mucosal incision is placed inferior to lip of sulcus and flap is developed. B,F) After similar step superiorly, sulcus (and contiguous cyst when present) is separated from vocal ligament (and vocalis if necessary) and excised. C,G) Flaps are further elevated to relieve tension and allow passive approximation. D,H) Further approximation is accomplished, if necessary, with one or two absorbable sutures.

and acoustic evaluations as previously described.[14] In addition to voice therapy, interventions included injections of collagen and steroids, medialization thyroplasty, incision with undermining, undermining with vertical slicing, and excision. Excised specimens were fixed and processed with hematoxylin and eosin stains. The most recent follow-up assessment was used for comparison with pretreatment studies.

TREATMENT METHODS

Voice Therapy. All patients exhibited glottic insufficiency that perhaps predisposed them to compensatory hyperfunctional activity. The goals of therapy were to encourage vocal hygiene, enhance breath support, and avoid hyperfunctional activity. Voice therapy was considered an important perioperative adjunctive measure.

Injections. Intracordal injections were performed with the orotracheal injector (Xomed-Treace, Jacksonville, Fla). Saline or 1% lidocaine hydrochloride with 1:10,000 epinephrine was injected to facilitate microdissection. Therapeutic injections of triamcinolone acetonide (10 mg/mL) was also performed intraoperatively in some cases. In an effort to correct bowing and to address scar tissue softening, cross-linked collagen (Zyplast, Collagen Corporation, Palo

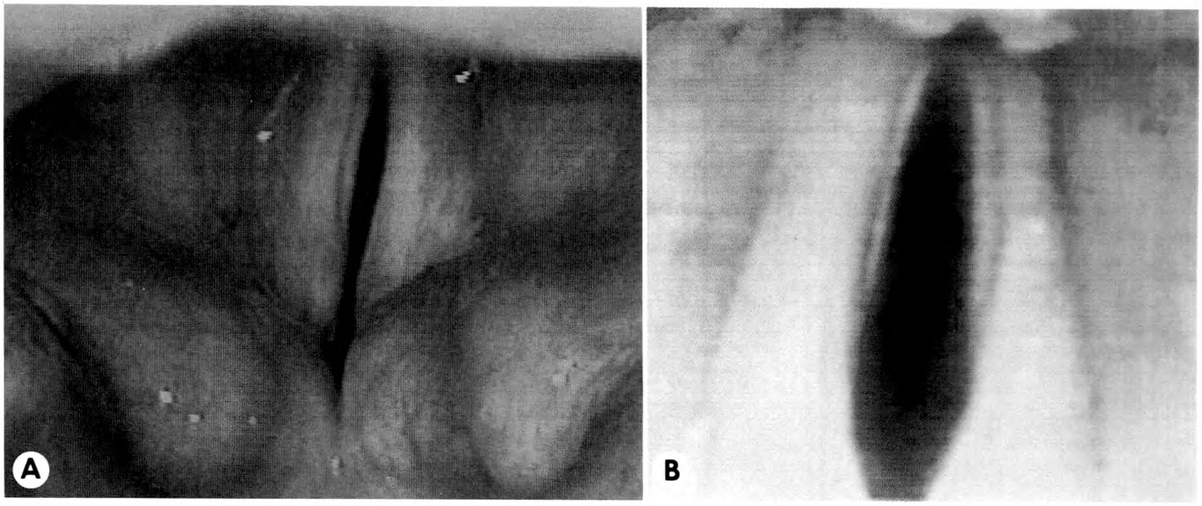

Fig 3. Physiologic sulcus in patient with vocal fold paralysis.

Alto, Calif) or, recently, autologous collagen (Autologen, Autogenesis, Acton, Mass) was injected into the most superficial layers accessible as previously described.[14] When injections failed to distribute appropriately, microincisions were placed to create a plane between the vocal ligament and covering epithelium. Neither Teflon nor fat injections were used to treat sulcus patients.

Incision and Undermining. Incisions were placed just superior to the edge of linear sulci, and the epithelium was sharply dissected from the vocal ligament. Additional undermining for a distance of 2 mm was done inferior to the sulcus to achieve adequate freeing of the mucosa. Neither suturing nor gluing was used with this technique.

Incision, Undermining, and Sectioning (Slicing). This technique, described by Pontes and Behlau,[17] is an extension of the incision and undermining approach just described. When the inferior flap has been undermined, a series of four or five incisions are placed in a vertical direction, producing three or four mucosal segments (Fig 1[17]). An important detail, illustrated but not discussed in the published description, is that each incision should be a different length (P. Pontes, personal communication, 1993); this impedes subsequent linear contracture. The superior surface mucosa is also undermined about 2 mm to help in redraping. The immediate postoperative appearance reveals denuded gaps along the medial edge; these heal remarkably well.

Excision. All excisions were done with microdissection techniques; laser was not employed; hemostasis was accomplished with topical 1:10,000 epinephrine. Incisions were placed to circumscribe the medial pore of the sulcus (Fig 2), and an inferior flap was developed. The superior approach was then used to dissect the sulcus away from the underlying vocal ligament, to which it was invariably adherent. Occasionally, sharp dissection into the vocal ligament with limited resection of the ligamentous structure was necessary. Even with extensive (2 to 4 mm) undermining, it is usually not possible to achieve good coaptation of the edges. When the residual gap was greater than 2 mm, a single 5-0 chromic catgut suture was placed (Fig 2H).

RESULTS

Patients with sulcus deformities of the vocal folds included those with apparently intact lamina propria without voice complaints at one end of the spectrum, and those with pathologic alteration of lamina propria and grossly disturbed voices at the other. Videostroboscopy was useful in distinguishing these two groups. The physiologic sulcus configuration group included patients with presbylaryngis, paralysis (Fig 3), and bowed vocal folds and a control group of volunteers with no specific voice complaints. A normal or minimally altered mucosal wave was characteristic of this latter group of patients. Table 2 shows the videostroboscopic findings in 9 patients, of the 116 with essentially normal voices, who exhibited physiologic sulcus and spindle-shaped glottal areas. In the most subtle cases, the sulcus was seen only on videostroboscopy during phonation, but in others it was apparent on indirect laryngoscopy during abduction. For purposes of further discussion, all of these patients will be considered as having type 1 or physiologic sulcus.

Diagnostic Assessment of Pathologic Sulcus Patients. Twenty other patients exhibited more advanced changes, with vocal fold stiffness (markedly reduced amplitude of vibration to complete absence of mucosal wave) and pathologic anatomic alterations of the vocal fold. Associated clinical findings

TABLE 2. VIDEOSTROBOSCOPIC FINDINGS IN CONTROL SUBJECTS WITH PHYSIOLOGIC SULCUS

Control Patient	*Sex*	*Age*	*Amplitude of Vibration*		*Mucosal Wave*		*Asymmetry*	*Closed Phase*
			R	*L*	*R*	*L*		
1	F	33	NL	NL	NL	NL	NL	NL
2	M	34	NL	NL	−1	−1	Minimal	NL
3	M	36	−1	NL	−1	NL	Minimal	−1
4	M	51	−1	−1	−1	−1	Minimal	NL
5	M	36	−1	−1	−1	−1	Minimal	−1
6	M	32	NL	NL	NL	NL	Minimal	−1
7	M	39	NL	NL	NL	NL	NL	NL
8	M	31	−1	−1	−1	−1	NL	−2
9	M	35	NL	NL	−1	NL	NL	NL

NL — normal limits. For amplitude of vibration and mucosal wave, −3 — markedly reduced, +3 — increased. For closed phase, −3 — predominantly open, +3 — predominantly closed.

included vocal fold edema, capillary ectasia, focal fullness, and vibratory disturbance (Table 3). These patients were considered candidates for surgical exploration and treatment. This pathologic sulcus group included two distinct types of lesions that were usually evident on videostroboscopy. One pattern was a linear sulcus along the medial vocal fold edge separating the superior and inferior lips of the membranous vocal fold by a rigid contracted band; this pattern is consistent with Bouchayer's description of a vergeture[2] and will be designated type 2 or sulcus vergeture (Fig 4A[2]). The other pathologic sulcus pattern was characterized by severely dysphonic voices in patients who might exhibit minimal abnormality on indirect laryngoscopy but demonstrated focal stiffness on videostroboscopy. Further examination occasionally revealed a medial pit-shaped sulcus that became obvious at microlaryngoscopy (Fig 4B). This type 3 sulcus or sulcus vocalis is the most specific pathologic sulcus designation.

Operative Observations. Microsurgical intervention was routinely preceded by careful examination with the operating microscope. Inspection of the

TABLE 3. LARYNGEAL FINDINGS IN OPERATIVE PATIENTS

Operative Patient	*Preoperative Clinical Findings*							*Additional Operative Findings*		
	Capillary Ectasia	*Edema*	*Bowing*	*Fullness*	*Mucosal Wave*	*Amplitude*	*Glottal Closure*	*Cyst*	*Microweb*	*Mucosal Bridge*
1				+	−1	−1	Incomplete		+	
2			+		−1	−1	Spindle			
3					−2	−2	Incomplete			
4			+		−2	−1	Incomplete			+
5	+	+		+	−2	−2	Posterior	+		+*
6			+		−2	−2	Spindle			
7			+		−2	−2	Longitudinal			
8			+		−2	−2	Spindle			
9			+		−2	−2	Incomplete			
10			+		−1	−1	Spindle			
11			+		−2	−2	Spindle	+	+	
12		+	+		−3	−2	Spindle			
13				+	−3	−3	Longitudinal	+		
14	+	+		+	−2	−2	Incomplete	+		
15	+				−3	−3	Posterior	+	+	
16			+		−3	−3	Posterior		+	
17	+		+		−2	−2	Posterior	+		+
18				+	−3	−2	Posterior	+		
19				+	−2	−2	Incomplete	+		
20			+	+	−3	−3	Spindle	+		

For mucosal wave and amplitude, −1 — mildly decreased, −2 — moderately decreased, −3 — severely decreased. For glottal closure, incomplete — incomplete closure, spindle — spindle-shaped gap, posterior — posterior gap, longitudinal — longitudinal gap.

*Multiple.

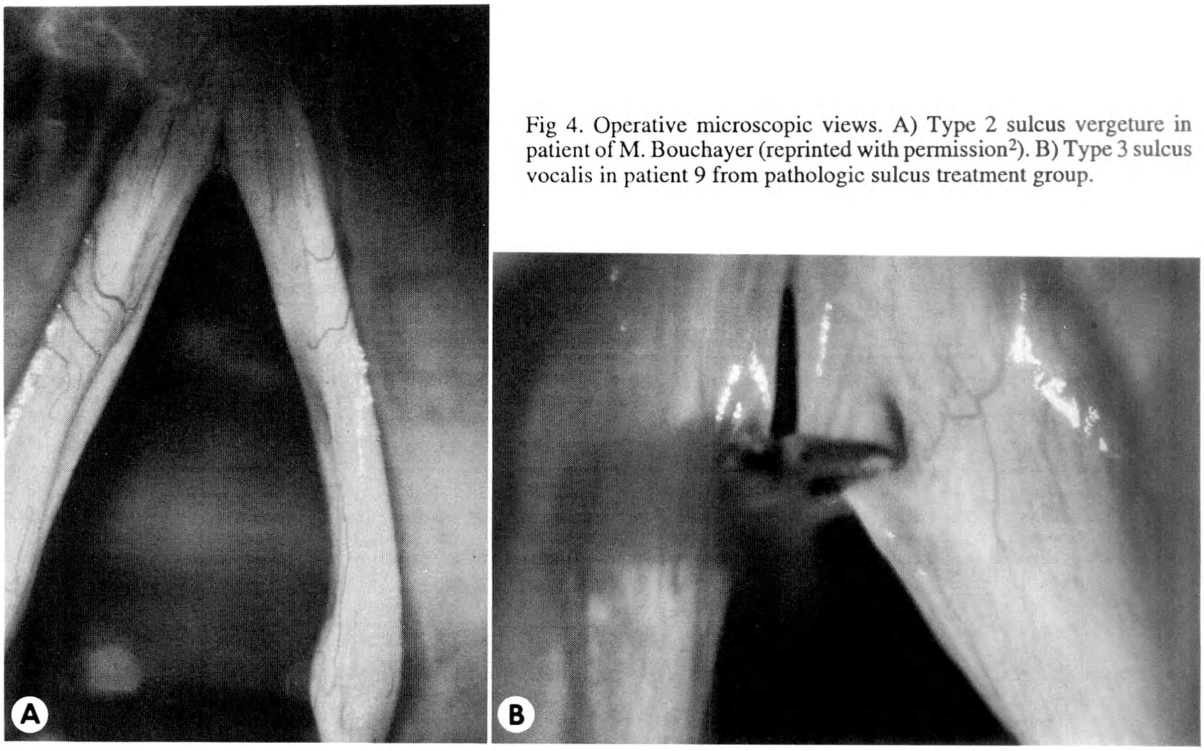

Fig 4. Operative microscopic views. A) Type 2 sulcus vergeture in patient of M. Bouchayer (reprinted with permission[2]). B) Type 3 sulcus vocalis in patient 9 from pathologic sulcus treatment group.

superior vocal fold surface often provided a clue as to the location and extent of intrafold pathologic changes. Pathologic sulci were invariably associated with prominent mucosal vessels or capillary ectasia on the superior surface of the vocal fold. Unlike the normal anterior-to-posterior–oriented vascular pattern, vocal folds with pathologic sulcus vocalis typically exhibited vessels branching at 90° angles and running perpendicular to the long axis of the vocal fold (Fig 4A).

Additional information found during microlaryngoscopy resulted from palpation and manipulation of the vocal fold. Palpation with a fine curved alligator forceps revealed focal induration, either distributed in a linear fashion along the medial vocal fold or associated with a focal invagination or pit. These deep pits could be displayed by grasping the inferior lip to distend the cavity (Fig 4B). The curved alligator forceps was also useful in probing the mucosal surface for mucosal bridges and in distending the anterior commissure to display associated microwebs. The associated lesions observed in this group of patients are tabulated in Table 3.

Histopathology. Specimens from this study were limited to those patients who underwent direct microlaryngoscopy with excision of the sulcus. One excised sulcus vergeture (type 2) demonstrated epithelial thinning and loss of the lamina propria cellular layered structure (Fig 5A[18]). The relationship of these pathologic changes to the underlying vocal fold structure can be appreciated in a whole-mount preparation (Fig 5B). These lesions demonstrated adherence of a thinned epithelium to the intermediate and deep layers of lamina propria, but there was no evidence of vocal ligament invasion, and there was minimal inflammatory response.

The focal type 3 sulcus vocalis lesions were associated with various degrees of inflammation. In some cases this was limited to a few inflammatory cells and increased capillaries (Fig 6A). In one case, in which the sulcus appeared to arise from a ruptured epidermoid cyst, there was intense inflammation with massive neovascularization and fibrosis extending through much of the vocal ligament (Fig 6B). In other instances, the sulcus was associated with intact epidermoid cysts. In contrast to the vergeture lesions, there was a tendency for these type 3 sulci and associated cysts to extend into the vocal ligament.

Results of Surgical Intervention. Initially we treated patients conservatively with voice therapy, collagen injections, and medialization thyroplasty. Voice improvements were limited in all of those early cases. Subsequent efforts involved direct surgical approaches to the affected vocal folds, with techniques varied depending on the disorder. Subjective improvements were much better in these 20 cases, and videostroboscopic findings confirmed improved glottal closure during phonation, improved vibratory activity, and better symmetry (Table 4). Objective measures confirmed voice improvement in most subjects (Fig 7). This was reflected in a reduction in perturbation measures (jitter and shimmer) and an

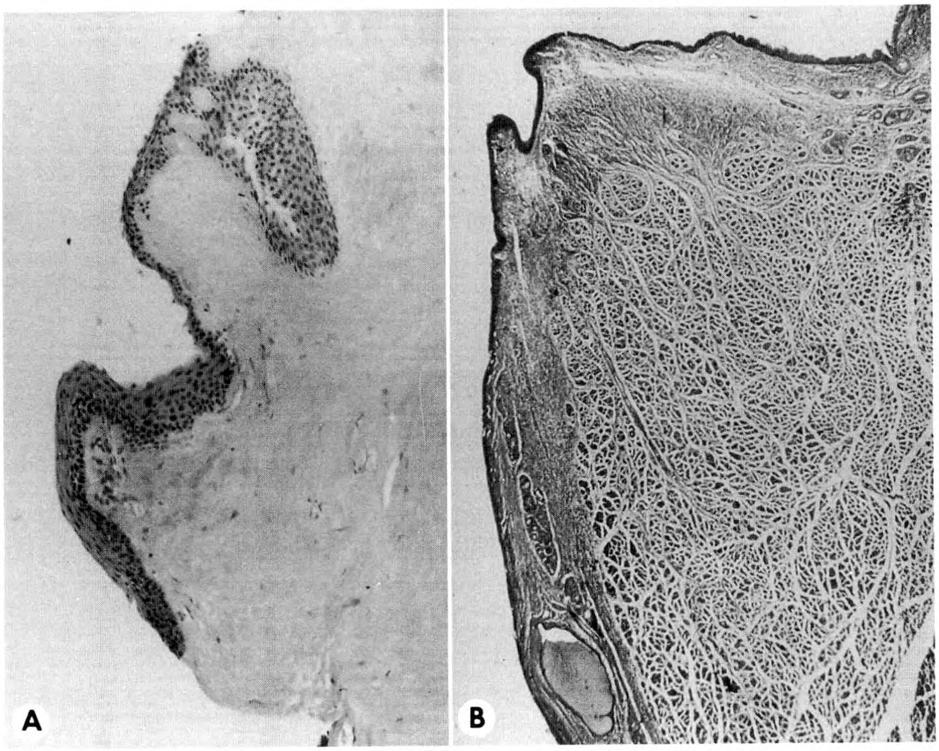

Fig 5. Sulcus vergeture. A) Photomicrograph of sulcus vergeture excised from patient 20 (H & E, original ×10). B) Whole mount laryngeal specimen from patient of M. Hirano (reproduced with permission[18]).

increase in signal-to-noise ratio. The voice frequency range was generally increased after intervention. Transglottic airflow was somewhat variable, but maximum phonation time tended to increase.

DISCUSSION

Making a meaningful diagnosis is essential to a substantive discussion of pathophysiology and pathogenesis, and to develop rational management strategies. It is not helpful to affix the sulcus vocalis label to all patients with vocal fold invaginations and spindle-shaped glottic configurations. The term sulcus literally means a depression or furrow and does not describe a pathologic entity. Such depressions on the medial surface of the vocal folds can occur in patients without vocal complaints, as in 9 of the 116

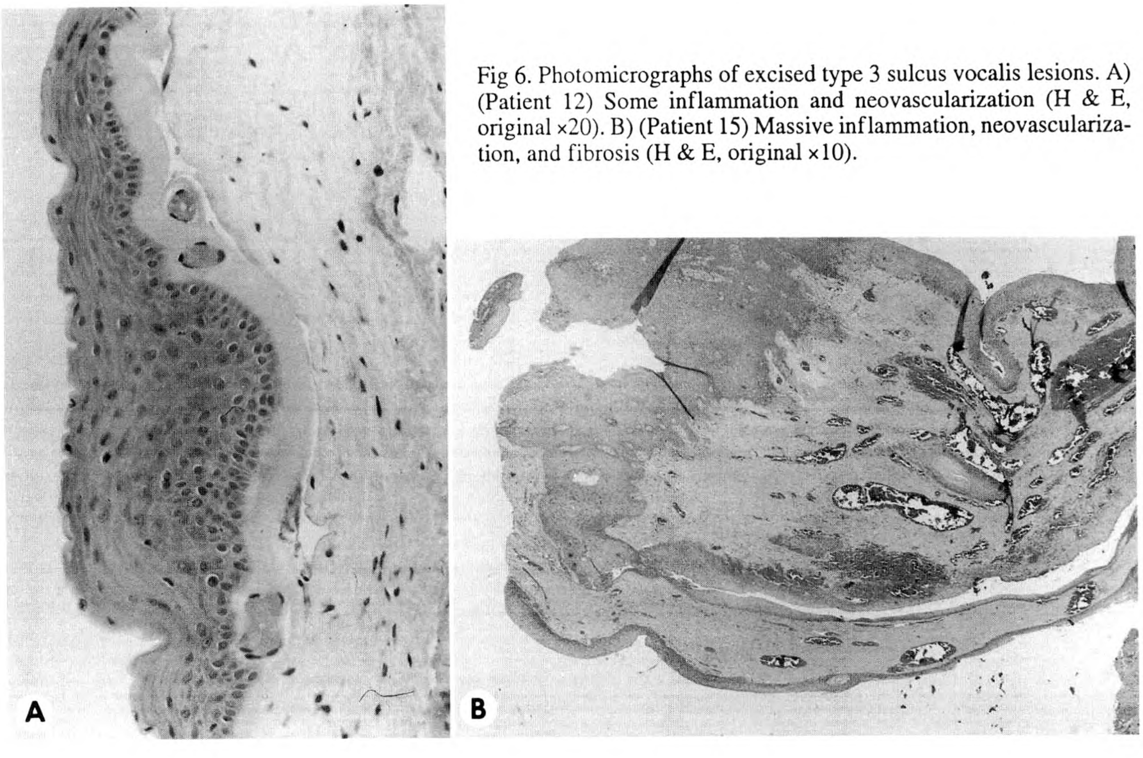

Fig 6. Photomicrographs of excised type 3 sulcus vocalis lesions. A) (Patient 12) Some inflammation and neovascularization (H & E, original ×20). B) (Patient 15) Massive inflammation, neovascularization, and fibrosis (H & E, original ×10).

TABLE 4. SURGICAL TREATMENT AND OPERATIVE RESULTS IN PATHOLOGIC (TYPES 2 AND 3) SULCUS PATIENTS

Operative Patient	Type of Operation		Type of Sulcus		Operative Results					
	R	L	R	L	Glottal Gap	Shape of Vocal Fold	Phase Closure	Amplitude	Mucosal Wave	Asymmetry
1	Und	Und	2	2						
2	Exc	Lysis + Col	2	2	Abs	Impr	Impr	Impr	Impr	Impr
3	Und	Exc	2	3						
4	Exc	Exc	3	3						
5	Exc	Exc	3	3	Abs	Impr	NC	Decr	Decr	NC
6	Exc	Exc	3	3	Decr	NC		Impr	Impr	
7		Lysis + Col	2	2	Decr	Impr	Impr	Impr	Impr	Impr
8	Lysis + Col	Col	2	2	Abs	Impr	Impr	Impr	Impr	NC
9	S	Und + S	2	2	Decr			NC	NC	
10	Und + S	Und + S	2	2	Incr	Impr	NC	NC	NC	NC
11	Exc	Und	3	2	Abs	Impr	NC	Impr	Impr	NC
12	Und	Exc	2	2	Decr	Impr	Impr	Impr	Impr	Impr
13	Exc	Exc	3	3	Decr	Impr	Impr	Impr	Impr	Impr
14	Exc	Exc	3	3						
15	Exc	Exc	3	3	NC	Impr	NC	Impr	Impr	NC
16	Und	Thy	2	2	Decr	Impr	Impr	Impr	Impr	NC
17	Exc	Exc	3	3	Abs	Impr		Impr	Impr	Impr
18	Slice	Und	2	2	Decr	Impr	Impr	Impr	Impr	Impr
19	Exc	Exc	3	3	Abs	NC	Impr	Impr	Impr	NC
20	Und	Slice	2	2	Abs	Impr	Impr	Impr	Impr	NC

For type of operation, Und — undermine, Exc — excision technique, Col — collagen injection, S — steroid injection, Thy — thyroplasty type I.
For type of sulcus, 2 — vergeture, 3 — vocalis.
For operative results, blank space — no data, Abs — absent, Impr — improved, NC — no change, Decr — decreased, Incr — increased.

volunteers reported here, and might be secondary to atrophic vocal fold changes from aging or paresis. These lesions, which we have designated as type 1 (physiologic sulcus), can be distinguished clinically by noting preservation of vocal fold vibratory activity on videostroboscopy. The presence of mucosal wave activity indicates adequate functional separation of the vocal fold body and cover as described by Hirano and Kakita.[5] The histologic correlate to such separation is an intact superficial lamina propria. It is important to understand that this population of sulcus patients exists and that it differs from the type 2 (vergeture) and 3 (vocalis) groups by having essentially intact layers of lamina propria. Considering any of these cases as sulcus vocalis might account for the great variability in the reported incidence of sulcus in voice-disordered patients.

Appropriate management of type 1 sulcus patients varies depending on the underlying disorder. In some instances it might be an incidental finding, and no treatment is necessary in such asymptomatic patients. When it is associated with paresis or loss of muscle bulk, voice therapy and possibly vocal fold medialization techniques might be helpful. As a general rule, it is preferable to avoid surgical disruption of the intact functioning membranous vocal fold tissues in these physiologic sulcus cases.

The hallmark of pathologic sulcus (types 2 and 3) is disturbance of the lamina propria, with loss of body-cover separation. This pathologic event disrupts normal vocal fold vibration and is manifested by stiffness on videostroboscopy and severe dysphonia. The 20 surgical patients reported here were similar in their clinical presentation: they had severe dysphonia, vocal fold stiffness, and incomplete glottic closure. The operative and pathologic findings indicated loss of superficial lamina propria and fixation of a thinned epithelium to underlying vocal ligament. In the type 2 sulcus vergeture group, the attachment was linear; in the focal type 3 sulcus vocalis, an epithelial-lined pit or contiguous cyst extended into or through the vocal ligament, evoking an inflammatory reaction.

Unlike the physiologic sulcus patients, pathologic sulcus patients required direct microsurgical inter-

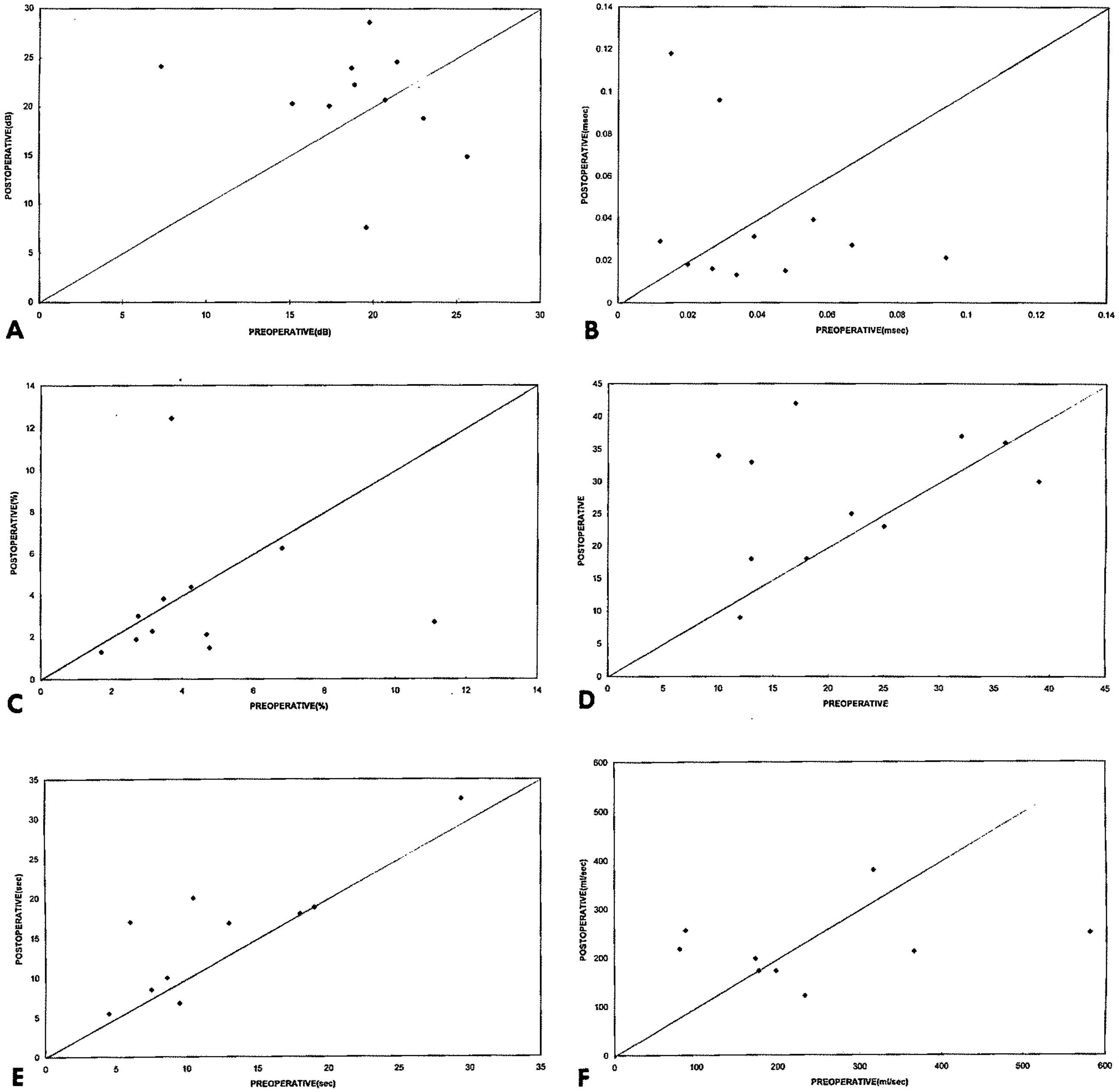

Fig 7. Scatter plots of patients who completed preoperative and postoperative voice assessment measures. Parameters include A) signal-to-noise ratio, B) jitter, C) shimmer, D) frequency range (semitones), E) maximum phonation time, and F) airflow. Values that are above diagonal indicate postoperative increased values, and those below diagonal show decreased values. Positive results were indicated by increased signal-to-noise ratio (A), frequency range (D), and phonation time (E). Decreases in jitter (B), shimmer (C), and airflow (F) were also considered favorable.

vention to achieve satisfactory results. Initial attempts to treat these lesions with injections and medialization yielded minimal improvement. Treatment that succeeded removed diseased tissue and achieved improved separation of covering epithelium from the vocal ligament and release of contracture. For type 2 sulcus vergeture lesions, the best results have occurred in 2 patients treated with the undermining and segmental slicing technique of Pontes and Behlau.[17] This approach employs the benefits of freeing mucosal attachments and also breaks up the linear contracture that divides the vocal fold. Postoperative videostroboscopy showed remarkable return of the mucosal wave and improved glottic closure in these cases. The spectacular results in this small series are insufficient to scientifically endorse such a radical-appearing approach, but they provide a rational approach to correcting the disorder.

The type 3 sulcus vocalis is the most demanding because of the interface of the lesion with remaining vocal fold structures. These sulci are typically associated with cysts, mucosal bridges, and/or considerable inflammation. Damaged fibrotic tissue and cystic remnants must be removed while preserving as much of the normal adjacent vocal fold tissue as possible. Once the lesion is excised, undermining of the residual mucosa facilitates coaptation and minimizes secondary-intention healing.

The proposed classification of sulcus deformities might facilitate treatment decisions and should allow investigators to compare results. The prevalence and pathogenesis of sulcus problems was not specifically addressed in this study. It is apparent that 116 volunteers were recruited as control subjects and 9 of these had evidence of physiologic sulci. However, this does not indicate an 8% prevalence, for many of these volunteers were concerned enough about their voices to seek a free voice evaluation, and several had minor laryngitis findings.

It remains uncertain as to whether pathologic sulci are congenital or acquired. The finding of associated epidermoid cysts in 9 of the 20 operative cases supports the hypothesis of Bouchayer et al[10] that these lesions are the result of ruptured congenital cysts. In 1 sulcus patient, the medially ruptured cysts showed extruding epithelial debris that was, on indirect laryngoscopy, suggestive of verrucous carcinoma. In this series, half of the patients reported onset of symptoms during childhood, which would support a possible congenital problem. In contrast, half of the patients reported adult onset of symptoms, and numerous potentially causative factors revealed in the histories of these patients included laryngitis, vocal abuse, allergy, and gastroesophageal reflux. The argument for acquired pathogenesis is also supported by our previous report of histologic sulci in laryngeal cancer cases showing that nearly half of the laryngectomy specimens contained adjacent histologic sulci.[4] The histologic findings in Fig 6B reveal an intense inflammatory response that was also characteristic of the reported cancer cases. Figure 6A shows a more extracellular response with loss of cellular detail. This type of response is similar to the superficial lamina propria responses that Gray et al[19] characterized by demonstrating increased fibronectin deposition and basement membrane zone injury. They postulated that such a pattern is a product of repetitive and chronic reinjury of the vocal folds leading to an aberrant healing response. Further study of the macromolecules that provide the filling around the delicate scaffolding of the superficial lamina propria (Reinke's space) might indicate a common pathway in the pathogenesis of sulcus vocalis that might explain how sulcus deformities are acquired from a variety of causes.

In summary, a series of patients with sulcus deformities of the vocal folds was presented. The clinical and pathologic findings indicate that some sulci are physiologic and can be observed in asymptomatic subjects. When there is damage to the superficial lamina propria, vocal fold vibration is impaired and patients become severely dysphonic. These cases respond favorably to microsurgery designed to remove destroyed tissue, release scar contracture, and promote mucosal redraping by regional undermining. Classifying cases into type 1 (physiologic sulcus), type 2 (sulcus vergeture), and type 3 (sulcus vocalis) should prove useful in understanding the pathogenesis, making treatment decisions, and comparing outcomes.

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Ann Otol Rhinol Laryngol 105:1996

TREATMENT OF HEAD AND NECK HEMANGIOMAS WITH RECOMBINANT INTERFERON ALPHA 2B

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Fifteen patients with head and neck hemangiomas were treated with systemic recombinant interferon alpha 2b (rIFNα2b, Schering). There were 14 infants and 1 adult in the group, ranging in age from 5 weeks to 24 years old. Of the 15 patients in the group, 5 had involvement of the airway. Three of the patients had previously failed alternative systemic therapy. Twelve patients have had a beneficial response. Ten patients have completed the therapy and have been off interferon from 6 to 53 months without reappearance or progression of the disease. Two patients are currently on the therapy with resolving lesions. Three patients had minimal response and underwent successful surgical resection. No major toxicity was encountered during the therapy. Our experience demonstrates that rIFNα2b is a well-tolerated and effective therapy for hemangiomas of the head and neck that require intervention.

KEY WORDS — hemangioma, interferon.

INTRODUCTION

Hemangiomas are the most common congenital lesion in infants and occur predominantly in the head and neck. An estimated 2.6% of all newborns present with hemangiomas, and 12% of all children at age 1 year have hemangiomas; hence, this lesion is the most common benign tumor of infancy.[1,2] Pathologically, hemangiomas consist of abnormal proliferations of endothelial cells. Histologically, this tumor is classified into three categories: capillary, cavernous, and mixed-type.[3] Most hemangiomas initially enlarge (proliferating phase) and then spontaneously regress, showing significant resolution by age 7 years.[1] Some lesions require intervention secondary to their large size, location near vital organs, activation of the clotting cascade with subsequent bleeding (Kasabach-Merritt syndrome), or development of congestive heart failure.

Therapeutic options have included surgery, steroids, embolization, laser surgery, sclerosis, chemotherapy, cryosurgery, and irradiation.[1,2,4-7] These therapeutic modalities carry a high risk of morbidity and often are of limited efficacy in treating life-threatening hemangiomas.[2,5,8] More effective and less toxic therapeutic modalities are needed.

Interferon alpha has recently been reported to be effective in the treatment of life-threatening hemangiomas.[8-15] We now report our experience in the treatment of 14 infants and 1 adult with head and neck hemangiomas using interferon alpha 2b.

METHODS

Patients. Patients were eligible for treatment if they had a histologic, radiographic, or clinical diagnosis of hemangioma. Patients also had to have tumors that required treatment secondary to deleterious physiological effects of tumors or severe disfigurement with functional compromise. Patients who were on additional therapy (ie, steroids) were weaned off these medications as soon as clinically permitted after treatment with interferon alpha began. Informed consent was obtained from the parents or the patient.

Treatment. Treatment consisted of interferon alpha 2b (rIFNα2b, Schering) at 3 × 10^6 IU per square meter of body area per day administered subcutaneously. If the platelet count was less than 20,000/mm^3 or if the patient had a bleeding disorder, rIFNα2b was given twice a day at 3 × 10^6 IU per square meter of body area (per dose) by intravenous injection. When the platelet count exceeded 20,000/mm^3 or the bleeding diathesis abated, once-a-day subcutaneous dosing resumed. Therapy was given daily for the first 6 months, then 3 times per week for an additional 6 months. Some patients were kept on therapy longer if clinically indicated.

Monitoring. Chemistry, hematology, and liver function tests were performed every 1 to 2 weeks initially and then monthly. Coagulation parameters were also monitored if disseminated intravascular coagulopathy (DIC) was present initially. Physical examination and developmental screening were performed every

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PATIENT CHARACTERISTICS AND OUTCOME

Patient	*Age*	*Sex*	*Locations*	*Complications*	*Previous Therapy*	*Duration of Therapy*	*Response**
1	1 mo	F	Neck and face, eyelid, parotid, pharyngeal, supraglottic, leg, palate	Disseminated intravascular coagulopathy, airway compromise, disfigurement, functional compromise	None	32 mo†	Major†
2	2 mo	M	Neck and face, lower lip, tongue, parapharyngeal, subglottic	Airway compromise, disfigurement, functional compromise	Tracheotomy	12 mo	Major
3	2 mo	M	Neck and face, eyelid, parotid, upper lip, subglottic	Airway compromise, disfigurement, functional compromise	Tracheotomy	14.5 mo	Major
4	6 mo	M	Neck and face, parotid, periorbital, ankle	Disfigurement, functional compromise	Steroids	14 mo	Major
5	4 mo	M	Maxillary sinus, retro-orbital, palate	Proptosis, failure to thrive	None	12 mo	Major
6	24 y	M	Supraglottic	Airway compromise, hemorrhage	Tracheotomy, laser	4 mo	Minimal, surgical resection
7	1.5 mo	M	Neck and face, thorax	Disseminated intravascular coagulopathy, disfigurement, functional compromise	Steroids, Amikar, dipyridamole, heparin	12 mo	Major
8	2.5 y	M	Tongue	Disfigurement, functional compromise	Steroids	10 mo	Minimal, surgical resection
9	1.5 y	F	Neck and face, lower lip, subglottic	Airway compromise, disfigurement, functional compromise	Tracheotomy	24 mo†	Major
10	2.5 mo	F	Eyelid	Disfigurement, functional compromise	None	12 mo	Major
11	2.5 mo	F	Retro-orbital	Proptosis	None	12 mo	Major
12	4 mo	F	Neck and face, back	Disfigurement, functional compromise, congestive heart failure	None	7 mo	Minimal, surgical resection
13	5 mo	F	Neck and face, parotid	Disfigurement, functional compromise	None	12 mo	Partial
14	10 mo	F	Neck and face, ear and parotid	Disfigurement, functional compromise	None	12 mo	Partial
15	3 mo	F	Neck and scalp, ear, parotid	Disfigurement, functional compromise	None	12 mo	Partial

*Major — ≥ 75% regression, partial — ≥ 25% but < 75% regression, minimal — 0% to 25% regression.
†Currently on therapy.

3 months during the therapy. Radiographic evaluations were also performed, if indicated, at enrollment and during follow-up.

Efficacy. Responses were assessed separately for the mass size and DIC. "Major response" was assigned when there was greater or equal to 75% reduction in the greatest dimension of tumor, "partial response" for greater than 25% but less than 75% reduction, "minimal response" for lack of progression of enlarging tumor and up to 25% reduction, "no response" for unchanged mass, and "progressive disease" for any enlargement. The tumor size was evaluated by physical examination and radiographic studies when indicated. "Major response" of DIC was assigned when all abnormal laboratory values normalized (ie, platelet count, fibrinogen). "Partial response" was an improvement, but continued abnormal laboratory values. "No response" was no improvement.

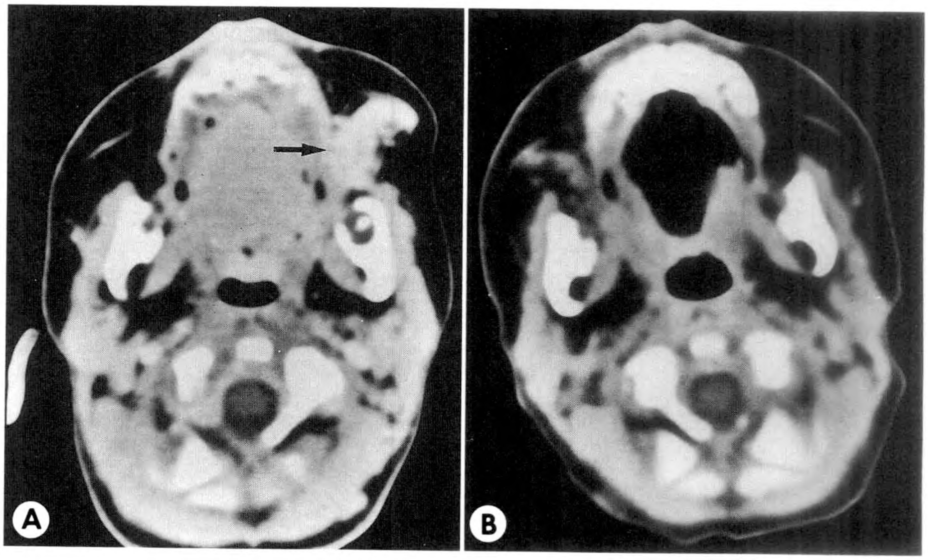

Fig 1. (Patient 5) Patient presented at age 4 months with failure to thrive, right orbital proptosis, and mass effect on right side of palate (arrow). Biopsy showed capillary hemangioma. A) Pretreatment computed tomogram. B) Posttreatment computed tomogram.

RESULTS

Fifteen patients with head and neck hemangiomas were treated by rIFNα2b: 14 infants and 1 adult (see Table). The age range for infants was from 5 weeks to 30 months, and the adult's age was 24 years. The most common involved area was the neck and face, followed by periorbital and retro-orbital soft tissues, periauricular soft tissues and auricle, airway, and oral structures. Eleven diagnoses were made on the basis of clinical and radiologic presentation, and 4 patients had biopsy-proven hemangiomas.

Therapy was initiated secondary to a variety of clinical complications, of which disfigurement with functional compromise was the most common (see Table). Other complications included airway compromise, Kasabach-Merritt syndrome, proptosis, failure to thrive, bleeding, and congestive heart failure. Three patients had failed steroid therapy, and 1, laser therapy. Four patients required tracheotomy secondary to respiratory distress. Twelve patients had no previous systemic therapy.

Good responses (major response or partial response) to the treatment were seen in 12 of the patients. Ten are off therapy from 6 to 53 months without reappearance or progression of hemangiomas. Two patients are currently on therapy with good responses. One of these 2 (patient 1) is back on therapy with a second excellent response after relapsing in the periauricular areas bilaterally 6 months after completion of interferon therapy. Representative examples of the results obtained are shown in Figs 1 and 2.

Three patients (2 infants and the adult) had minimal response and successfully underwent surgical resection. The 2 patients with Kasabach-Merritt syndrome both showed complete resolution of their DIC. Three infants with subglottic hemangiomas had resolution of the lesions. One has been decannulated and the other 2 await decannulation.

Toxicity was mild and of short duration, consisting of fever, nausea, mild elevation of liver enzymes, and neutropenia. In all cases toxicity resolved, with no patients requiring discontinuation of therapy due to toxicity. One patient had the dose of interferon temporarily adjusted for elevation in liver enzymes and was able to resume full-dose therapy and finish treatment as planned after liver function improved. Height, weight, and development have been periodically monitored during therapy and in follow-up, and have remained normal in all patients compared to national norms.

DISCUSSION

Hemangiomas are benign tumors that represent an arrest in development of the vascular system in the capillary network or retiform stage.[1,3] Their natural history is rapid growth in the first few years of life (proliferating phase) and then spontaneous regression in the next 5 to 8 years (inactive phase).[1,8] Greater than 50% of hemangiomas show complete resolution by the age of 5 years, and greater than 70%, by the age of 7 years, with continued slow regression of the remaining lesions by the age of 10 to 12 years.[1,8] Secondary to this fact, it is important to be cautious in evaluating any therapeutic intervention. However, spontaneous regression of lesions prior to age 1 year is unusual.[9]

While the majority of hemangiomas are best managed by observation, some require immediate intervention because they undergo rapid growth, endangering life or affecting organ systems. Treatment modalities that have had variable success include cryosurgery, irradiation, chemotherapy, embolization,

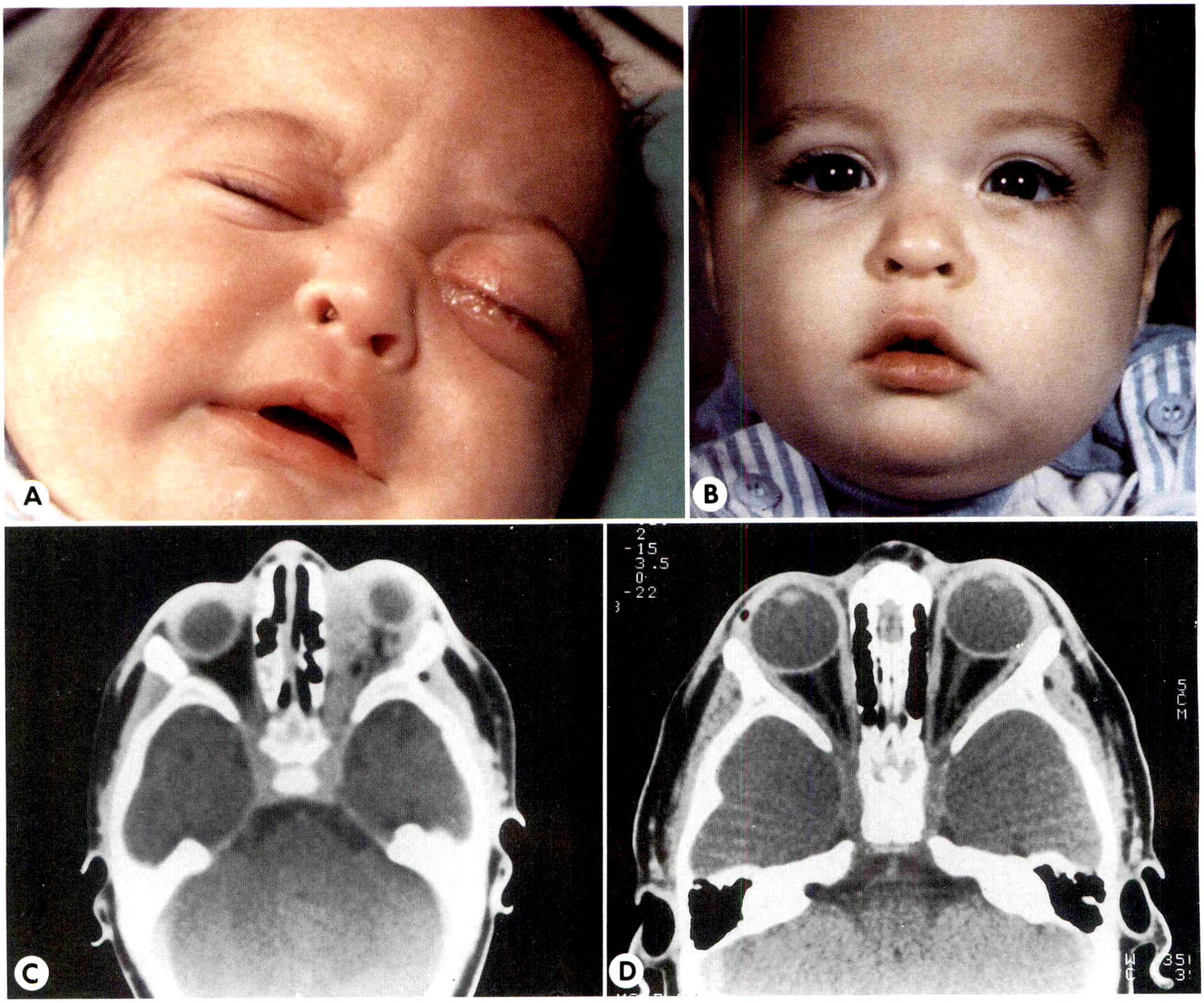

Fig 2. (Patient 11) Patient presented with left orbital proptosis. Biopsy showed capillary hemangioma. A) Pretreatment photograph. B) Posttreatment photograph. C) Pretreatment computed tomogram. D) Posttreatment computed tomogram.

and sclerosing agents.[1,2,4-7] These treatment options have been associated with high morbidity, especially scarring, tissue necrosis, and infection. Irradiation and chemotherapy have additional potential for long-term complications, most significantly secondary malignancy and inhibition of growth.[1,2]

Traditional surgical excision, with or without preoperative embolization, may be indicated in cases of heart failure secondary to arteriovenous shunts, visual or luminal obstruction, and uncontrolled bleeding or infection.[1,2,6,7] Often tumor location or risk of complication precludes surgical intervention or embolization.

The carbon dioxide laser has been shown to be successful in treatment of isolated, unilateral subglottic hemangiomas.[7] Involvement of multiple organ systems and/or extension of lesion beyond the larynx and trachea precludes use of the laser.[2,6,7,9] However, subglottic stenosis has been reported as a complication of its use, especially in cases of circumferential lesions and repeat applications.[9]

Medical therapy with corticosteroid has been traditionally used for life- or organ-endangering hemangiomas.[2,4,5] Steroids are believed to act by blocking estradiol-17 beta receptors in the cytoplasm of proliferating endothelial cells in hemangiomas. Also, it has been theorized that steroids block mast cells and release of local growth factors that are believed to play a role in the proliferating phase.[1] Multiple growth factors such as epidermal growth factor, transforming growth factor beta, platelet derived growth factor, and fibroblast growth factor have been shown to have angiogenic effects.[16] The reported response to steroids has been highly variable and associated with significant growth delay and toxicity.[2,5,8] Enjolars et al[5] reported a 30% response to prednisone in a group of patients with life-threatening hemangiomas. Prednisone treatment had questionable value in 40% of patients and elicited no response in 30% of them. Because of the high morbidity and limited efficacy of conventional therapies, alternative therapies for treatment of hemangiomas are necessary.

One of the newest and most exciting approaches is the use of interferon alpha. In 1989, Orchard et al[10] reported successful treatment of 2 children with hemangiomas by rIFNα2b. White et al[11-13] reported similar results using interferon alpha 2a. Ezekowitz et al[8] reported the first large series of 20 infants treated for life-threatening hemangiomas with interferon alpha 2a; in this report 18 of 20 patients had 50% or more regression of their lesion with minimal side effects. Ohlms et al[9] reported the first large series of 15 infants treated for life-threatening airway hemangioma using interferon alpha 2a, with excellent results in all patients and no long-term complications. Ricketts et al[14] and Loughnan et al[15] have also reported successful treatment of massive capillary hemangiomas with alpha interferon.

We hereby report on 15 patients with head and neck hemangiomas who were treated with rIFNα2b. Twelve of 15 patients have had major or partial responses, requiring no additional treatment modality. Two infants are still on treatment from this group.[1] Both patients with Kasabach-Merritt syndrome showed complete resolution of their DIC.

Duration of therapy was initially chosen arbitrarily and based primarily on clinical response. At the present time, prolonged therapy (minimum of 12 months) is advocated if there is good clinical response, since the majority of patients who completed the protocol did not relapse. Relapse in patient 1 after successful completion of therapy, with regression of the recurrent lesions on reinstitution of interferon, suggests that it may be desirable to continue the interferon therapy as long as lesions are in the proliferating phase. Further studies are indicated to optimize the duration of therapy.

The mechanism of action of alpha interferon on the hemangiomas is still unknown. We have postulated that the interferon has a negative effect on the growth of endothelial cells within hemangiomas during their proliferative phase. The original hypothesis leading to the application of interferon in this clinical setting was based on several key observations: interferon alpha has a 30% to 50% response rate in the treatment of Kaposi's sarcoma, which is an endothelial cell tumor; interferons inhibit endothelial cell migration and proliferation, and other steps in angiogenesis, in vitro; interferons inhibit tumor or white blood cell–induced angiogenesis; interferons inhibit the effect of growth factors such as basic fibroblast growth factor and/or are mutually antagonistic to growth factors.[17-23]

Our experience using rIFNα2b in the treatment of complex hemangiomas of the head and neck has demonstrated excellent results with consistent stabilization of lesions and accelerated involution. Therapy was well tolerated, and the follow-up to this date has shown no long-term side effects and normal growth and development.[1] The response to therapy has allowed patients to avoid the complications of heroic interventions. This has allowed time to achieve maximal regression prior to surgical intervention if deemed necessary, maximizing the potential to reach a successful functional and cosmetic result.

CONCLUSION

Interferon alpha 2b is an effective and well-tolerated therapy for head and neck hemangiomas requiring intervention when conventional treatments fail. Further studies are needed to determine optimal dosing, duration of therapy, and possible long-term side effects. A randomized controlled comparison between alpha interferon and systemic steroids is needed to better define the relative roles for these therapies.

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LARYNGEAL ELECTROMYOGRAPHY FINDINGS IN IDIOPATHIC CONGENITAL BILATERAL VOCAL CORD PARALYSIS

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Children with idiopathic congenital bilateral vocal cord paralysis (BVCP) were investigated by electromyography (EMG) of the posterior cricoarytenoid and thyroarytenoid muscles to determine whether laryngeal EMG findings had diagnostic or prognostic significance. Four children between 3 weeks and 33 months of age were studied. Three had abductor paralysis and were tracheostomy-dependent, while the fourth had adductor paralysis requiring a feeding gastrostomy. Two of these patients also had other anomalies. Motor unit potentials showing phasic bursts with respiration were found in all four cases, while three children developed a full interference pattern on lightening of the anesthetic. Follow-up for between 37 and 52 months showed no significant clinical improvement in any of the patients. While the diagnosis of idiopathic congenital BVCP can represent a heterogeneous group of conditions, the findings suggest that normal laryngeal EMG findings may be a feature of idiopathic congenital BVCP but do not imply a favorable prognosis for early recovery. They may, however, have implications to explain the likely site of lesion in idiopathic congenital BVCP.

KEY WORDS — laryngeal electromyography, vocal cord paralysis.

INTRODUCTION

Vocal cord paralysis is regarded as the second commonest cause of stridor in neonates.[1] Vocal cord paralysis may be unilateral or bilateral; however, these two conditions tend to differ with respect to both their clinical presentation and their underlying cause.

Typically, congenital bilateral vocal cord paralysis (BVCP) presents with symptoms of significant upper airway obstruction soon after birth. The vocal cords lie close to the midline and fail to abduct, or abduct inadequately with inspiration, a condition generally referred to as abductor paralysis. Phonation is usually normal. Less commonly, vocal cord dysfunction associated with upper airway obstruction may be due to incoordinated vocal cord movement in which vocal cord abduction occurs randomly or at an inappropriate time in the respiratory cycle.

Rarely, the vocal cords may fail to adduct and lie in an abducted position, and present with features of laryngeal incompetence without symptoms of upper airway obstruction. In a review of pediatric vocal cord paralysis, Swift and Rogers[2] reported 13 children with BVCP, 2 of whom had partial adductor paralysis. Mace et al[3] reported a family with isolated hereditary bilateral adductor paralysis affecting 5 individuals over three generations, with symptoms being present at birth in 4 cases.

A case of BVCP may be partial or complete, with the severity of bilateral abductor paralysis of the vocal cords being usually gauged by the degree of upper airway obstruction present and whether or not a tracheostomy is required. Other factors may, however, influence the decision to perform a tracheostomy, besides simply the size of the glottic airway during inspiration.

Holinger et al,[4] in a review of 149 children with BVCP, found 71 (48%) who required tracheostomy. In a series of Cohen et al,[5] 45 of 62 children (73%) with BVCP required tracheostomy, and a number of smaller series reported tracheostomy rates varying from 54% to 92%.[2,6-8]

Impairment of vocal cord mobility is best determined by flexible laryngoscopy in the awake patient.[1] This subjective evaluation, particularly in newborns, makes detection of subtleties of vocal cord dysfunction quite difficult and somewhat imprecise. As a consequence, it may not be possible to determine purely by endoscopic means whether or not vocal cord paralysis is partial or complete and whether the paralysis is exclusively unilateral or there is also a less severe degree of involvement of the contralateral vocal cord present in addition.

The prognosis for recovery of vocal cord function depends on the underlying cause of the congenital BVCP. After excluding structural laryngeal abnormalities that limit vocal cord mobility, the causes of congenital BVCP that need to be considered are birth trauma or asphyxia and central nervous system anomalies, most commonly the Arnold-Chiari malforma-

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tion. Other structural neurologic abnormalities have also been described, and these include hydrocephalus unrelated to Arnold-Chiari malformation, hypoplasia of cranial nerve motor neurons, and cerebral agenesis.[4] In some cases, the cause of congenital BVCP may not be readily apparent.

Congenital BVCP may be hereditary, and a number of family pedigrees have been reported that include individuals with isolated vocal cord paralysis and those in whom other anomalies were also present that generally involved neurologic and musculoskeletal disorders.[3,4,9-19]

In their review of BVCP in children, Holinger et al[4] found 71 of 114 cases of congenital BVCP in which the cause was unknown. In 32 of these, other anomalies were also found that included neurologic, cardiac, and laryngeal abnormalities. Emery and Fearon[8] found 10 of 19 children with congenital vocal cord paralysis in whom the cause was unknown, and 5 of these had associated anomalies. It would appear that in a significant proportion of cases of congenital BVCP, no cause may be found. In addition, it seems appropriate to categorize patients with idiopathic congenital BVCP into two groups, those with and those without associated anomalies.

The site of lesion in idiopathic congenital BVCP, which is assumed to lie at or above the level of the laryngeal brain stem motor neurons, is unknown. It would seem unlikely, however, that there is a single pathologic abnormality that is common to all cases of idiopathic congenital BVCP — a fact underscored by the variations in clinical presentation of this condition.

While the outcome of congenital BVCP can be predicted on the basis of the underlying cause of the condition, the prognosis for recovery when no cause is found, particularly in severe cases of BVCP, ie, those requiring tracheostomy, is unknown. Cohen et al[5] followed up 14 children with idiopathic congenital BVCP, 10 of whom had required a tracheostomy, to a mean age of 76 months. They found complete resolution in 4, partial resolution in 7, and no improvement in 3. Emery and Fearon[8] observed spontaneous recovery in 2 of 5 children, and Gentile et al[7] found recovery in both of their 2 children, at 6 months and 5 years, respectively.

In an attempt to improve understanding of the pathogenesis of idiopathic congenital BVCP and determine prognostic factors for recovery, laryngeal electromyography (EMG) was performed on a group of children with severe congenital BVCP of unknown cause, followed by monitoring of their subsequent clinical progress.

METHODS

All 4 children had BVCP from birth that required early surgical intervention. The diagnosis of BVCP was made by flexible laryngoscopy performed by the author with the patient awake, and was confirmed by microlaryngoscopy and bronchoscopy under general anesthesia. This examination included assessment of passive mobility of the vocal cords. Cases involving vocal cord fixation or birth asphyxia or trauma, or in which a significant structural abnormality of the central nervous system was found, were excluded.

Laryngeal EMG was not performed as an isolated procedure, but was carried out at the same time as either tracheostomy or endoscopic evaluation of the airway.

Laryngeal EMG was performed under general anesthesia with the patient breathing spontaneously on oxygen and halothane administered through a tracheostomy tube when present, or otherwise via face mask initially and then by a nasopharyngeal catheter. No topical anesthetic was used. The larynx was visualized either by means of a handheld Magill laryngoscope or by suspension microlaryngoscopy. A concentric needle electrode was used, and insertion was attempted in the right and left posterior cricoarytenoid and thyroarytenoid muscles.

Motor unit potentials were recorded with a Medelec Mystro electromyograph following lightening of the anesthetic and also tracheostomy tube occlusion. A capnograph was used to simultaneously record respiratory activity.

The EMG was interpreted by a pediatric neurologist, with a normal graph being regarded as one in which normal motor unit potentials were recorded without evidence of fibrillation potentials, positive sharp waves, or complex repetitive discharges. The motor unit potentials showed phasic bursts with inspiration on tracheostomy occlusion, and a full interference pattern in response to lightening of the anesthetic.

Subsequently, the patients were followed up by awake flexible laryngoscopy performed by the author. In children with abductor paralysis of the vocal cords, cord mobility was assessed by flexible laryngoscopy during tracheostomy tube occlusion.

RESULTS

Four children with idiopathic congenital BVCP, between 3 weeks and 33 months of age, underwent laryngeal EMG. Their details are listed in the Table.

Three children had abductor paralysis and had required a tracheostomy within the first few weeks of

DETAILS OF PATIENTS WITH IDIOPATHIC CONGENITAL BILATERAL VOCAL CORD PARALYSIS WHO UNDERWENT LARYNGEAL ELECTROMYOGRAPHY

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*
Gender	Female	Male	Female	Male
Birth history	Term, elective cesarean section	Term, vaginal delivery, no forceps	Term, vaginal delivery, no forceps	Term, vaginal delivery, no forceps
Diagnosis	Bilateral abductor paralysis	Bilateral abductor paralysis	Bilateral abductor paralysis	Bilateral adductor paralysis
Treatment	Tracheostomy at 13 d	Tracheostomy at 21 d	Tracheostomy at 3 d	Gastrostomy at 4 mo
Associated anomalies	None	None	Facial muscle hypoplasia, mild developmental delay	Dysmorphic features, unilateral choanal atresia, submucous cleft palate, atrioseptal defect, developmental delay
Neurologic investigations	Ultrasound and CT normal	No investigations recommended by neurologist	Ultrasound normal, CT showed mildly dilated ventricles with unknown significance	Ultrasound normal, CT showed lateral ventricles to be dysplastic
EMG: age	3 mo	3 wk	2 y 9 mo	7 mo
EMG: weight	4.3 kg	3.4 kg	12.8 kg	4.5 kg
EMG: other procedures	Microlaryngoscopy, bronchoscopy	Microlaryngoscopy, bronchoscopy, tracheostomy	Microlaryngoscopy, bronchoscopy	Microlaryngoscopy, bronchoscopy
EMG: muscles tested	R and L thyroarytenoid, R and L posterior cricoarytenoid	R and L posterior cricoarytenoid	R and L thyroarytenoid, R and L posterior cricoarytenoid	R thyroarytenoid
EMG: results				
Motor unit phasic bursts	Yes	Yes	Yes	Yes
Development of full interference on lightening anesthetic	Yes	Yes	Yes, but reduced interference in posterior cricoarytenoid	Not attempted
Follow-up period	3 y 8 mo	3 y 10 mo	3 y 1 mo	4 y 4 mo
Outcome	No purposeful abduction	Some abduction, inadequate lumen	No purposeful abduction	Some purposeful adduction

CT — computed tomography, EMG — electromyography.

life. Two of these had BVCP as an isolated anomaly, and both showed normal motor unit potentials with phasic activity and increased recruitment to full interference pattern with lightening of the anesthetic and tracheostomy tube occlusion (Figs 1 and 2).

One child had abductor paralysis in association with facial muscle hypoplasia and mild developmental delay. She subsequently developed a number of acquired airway abnormalities, including an interarytenoid web and tracheomalacia. Assessment of vocal cord mobility under anesthesia following division of the web showed normal mobility. Her laryngeal EMG showed a full interference pattern, but with reduced interference in the posterior cricoarytenoid, suggestive of a decreased number of functioning motor units (Fig 3).

A fourth child had marked laryngeal incompetence due to bilateral adductor paralysis and required a feeding gastrostomy. In addition, this was associated with dysmorphic features, unilateral choanal atresia, submucous cleft palate, atrioseptal defect, and significant developmental delay. Chromosome study findings in this child were initially reported as normal, but a repeat test showed interstitial deletion of chromosome 22 with karyotype 46,XY, del(22)(q11.22q12.2). Only a limited study was possible in this patient. Normal motor unit potential morphology was demonstrated in the right thyroarytenoid muscle, but the response to lightening of the anesthetic could not be evaluated (Fig 4).

There was no clinically significant improvement in any of the 4 cases during a period of follow-up ranging from 37 to 52 months, with the patients remaining tracheostomy- or gastrostomy-dependent. There was, however, some observable improvement in vocal cord movement in 2 cases. The 1 child with abductor paralysis who improved failed a trial of decannulation recently, despite undergoing repeat microlaryngoscopy and bronchoscopy that showed a

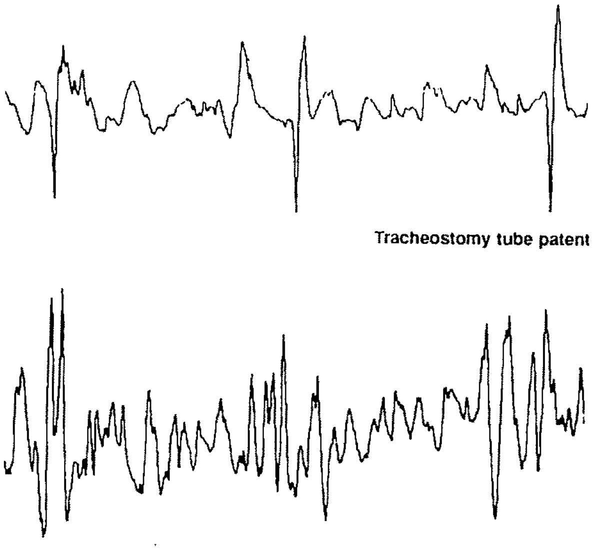

Fig 1. (Patient 1) Motor unit potentials in right posterior cricoarytenoid muscle with development of full interference pattern following tracheostomy tube occlusion.

normal airway with normal passive mobility of the vocal cords.

DISCUSSION

The motor unit is an anatomic unit composed of a motor neuron, its axon, the neuromuscular junctions, and all the muscle fibers innervated by the axon. The motor unit action potential is the compound muscle action potential of those muscles within the recording range of the needle electrode. The motor unit action potential is characterized by amplitude, duration, number of phases, and stability of the configuration with consecutive discharges. As the strength of voluntary contraction is increased, there is an orderly recruitment of additional motor units. The configurations and recruitment pattern of motor unit action potentials can be assessed qualitatively and measured quantitatively in EMG studies.[20]

With regard to EMG studies in general, the motor unit action potential parameters in infants, children, and adults have been found to be quite similar, with an increase in amplitude and duration of only 20% being documented between the ages of 3 months and 20 years, despite the difference in size of the muscles.[20]

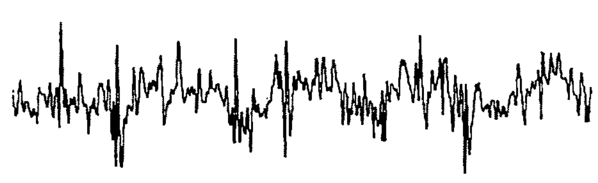

Fig 2. (Patient 2) Motor unit potentials in right posterior cricoarytenoid muscle with marked increase in interference pattern following lightening of anesthetic.

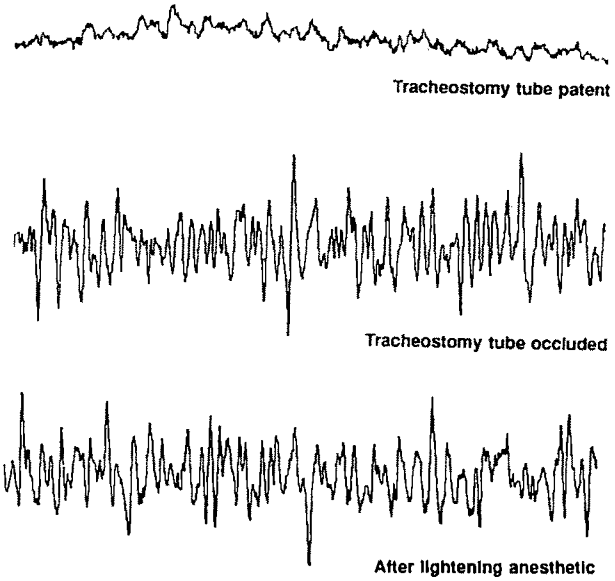

Fig 3. (Patient 3) Motor unit potentials in right thyroarytenoid muscle showing response to tracheostomy tube occlusion and lightening of anesthetic.

Electromyography of the intrinsic muscles of the larynx has been found to be a useful clinical tool in the evaluation of vocal cord function. It can be used to differentiate between structural and neurologic causes of vocal cord immobility, to localize the site of a neuromuscular lesion, and to predict the likelihood of recovery following nerve injury.[21] Laryngeal EMG is widely employed in the assessment of vocal cord function in adults; however, there is a paucity of information available regarding laryngeal EMG studies in children, with only two reports having been published on this subject.

Using monopolar EMG, Koch et al[22] studied the posterior cricoarytenoid muscle in 12 children with vocal cord paralysis, 5 of whom had congenital paralysis. In the congenital paralysis group 2 children were found to have unilateral vocal cord paralysis on the basis of demonstrating only occasional motor unit potentials on one side and essentially normal motor unit potentials on the other side. Two children with associated congenital anomalies both showed either absent or only occasional motor unit potentials; this finding was interpreted as confirming the diagnosis

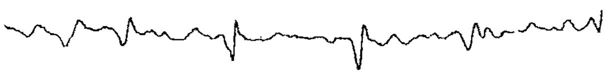

Fig 4. (Patient 4) Isolated motor unit potentials in right thyroarytenoid muscle showing normal morphology and some bursting with respiration.

of vocal cord paralysis. In 1 child, born premature and formerly tracheostomy-dependent with documented subglottic stenosis, the presence of motor unit potentials was interpreted as indicating a mechanical rather than a neurologic cause of obstruction.

Gartlan et al[23] used percutaneous bipolar hooked-wire electrode EMG to study the thyroarytenoid muscle in 4 children with vocal cord paralysis, 1 of whom had symptoms from the time of birth. This infant, who was otherwise well, had an immobile right vocal cord and paresis of the left vocal cord. Laryngeal EMG showed some motor unit activity on the left, but none on the right; this finding was thought to indicate bilateral recurrent laryngeal nerve injury with only partial injury on the left.

Our study of 4 children with severe idiopathic congenital BVCP has documented the presence of motor unit potentials in the posterior cricoarytenoid muscle of 3 children with abductor paralysis, and in the thyroarytenoid muscle of 1 patient with adductor paralysis. Phasic bursts with respiration were observed in all children, and in 3 a full interference pattern with lightening of the anesthetic was found.

Although only 4 children have been studied, the data obtained by laryngeal EMG were relatively consistent, and redefine our understanding of idiopathic congenital BVCP with regard to diagnosis, prognosis, and underlying pathogenesis.

The presence of normal electrical activity in laryngeal muscles despite an apparent lack of muscle function suggests that normal laryngeal EMG findings may be a characteristic feature of idiopathic congenital BVCP. Laryngeal EMG, therefore, cannot be used to differentiate between a mechanical cause of vocal cord dysfunction and idiopathic congenital BVCP.

Furthermore, normal laryngeal EMG findings in the presence of a diagnosis of idiopathic congenital BVCP have no prognostic implications. The 4 children in this study, all of whom had severe disease requiring surgical intervention, showed no significant clinical improvement (although there was some observable improvement in vocal cord mobility in 2 cases) over a period of follow-up ranging from 37 to 52 months. Regular follow-up by microlaryngoscopy and bronchoscopy with evaluation of passive vocal cord mobility was not routinely performed, however, and the contribution made toward the lack of clinical improvement by vocal cord fixation occurring subsequent to the initial assessment cannot be adequately determined.

This study also has implications regarding the site of lesion in idiopathic congenital BVCP. Normal laryngeal EMG findings suggest that the motor unit is intact, and in general rule out a lesion being present anywhere from the motor neuron to the muscle fiber.

The cause of vocal cord immobility in idiopathic congenital BVCP is unclear, but could be explained on the basis of the laryngeal EMG findings by inappropriate muscle activity due to a lack of coordination between the actions of the laryngeal abductors and adductors with respect to the respiratory cycle, leading to sustained vocal cord adduction during inspiration in the case of abductor paralysis. This would suggest as a possible site of lesion the ventrolateral medulla, where the laryngeal motor neurons are situated and receive afferents from respiratory neurons. Alternatively, idiopathic congenital BVCP could be due to a muscle disorder, primarily involving either the force of contraction or electrical-mechanical coupling.

The 4 cases reported showed similarities in laryngeal EMG findings and prognosis, despite the variation in clinical presentation. It would seem appropriate to classify idiopathic congenital BVCP clinically into those cases with and those without associated anomalies. In the latter group, findings on cranial ultrasound and computed tomography are likely to be normal, but the value of more sophisticated investigations such as magnetic resonance imaging cannot be determined from this study.

The presence of associated anomalies should, however, prompt a genetics consultation and chromosome studies. Although the findings on chromosome analysis were initially reported as normal in patient 4, a repeat test showed an interstitial deletion of chromosome 22, which appears to explain the majority of the patient's features but not specifically the vocal cord paralysis.

Chromosomal abnormalities in children with vocal cord paralysis have previously been reported by Emery and Fearon,[8] who cited 2 cases of translocation of part of chromosome 14, and Holinger et al,[4] who reported 2 cases of Down's syndrome and 1 case in which there was an abnormality of the short arm of a D chromosome.

In conclusion, while the clinical presentation of idiopathic congenital BVCP can be variable, the condition appears to be characterized by normal findings on laryngeal EMG, but this does not have any prognostic implications. The site of lesion remains unclear, but normal laryngeal EMG findings suggest that idiopathic congenital BVCP may be due to an imbalance between the actions of the laryngeal abductors and adductors, rather than being a consequence of true laryngeal muscle paralysis.

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Ann Otol Rhinol Laryngol 105:1996

ANALYSIS OF T CELL RECEPTOR β CHAIN REPERTOIRE IN MIDDLE EAR EFFUSIONS

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In order to elucidate the immune response in otitis media with effusion (OME), the polymerase chain reaction was employed to examine T cells in middle ear effusions in patients with OME for utilization of T cell receptor (TCR) variable region genes. Specimens of RNA were extracted from 13 ears of 12 patients (9 children and 3 adults). Oligonucleotide primers specific for individual TCR Vβ gene families were used to amplify TCR gene products in each sample. Although the number of Vβ families utilized by each sample varied from 1 family to 21, a few significant trends emerged. Eleven ears out of 13 expressed Vβ7, which was the most frequently utilized (84.6%) Vβ family among the 24 Vβ families. In 5 of the 13 samples, the number of Vβ families utilized was restricted to 1, which was Vβ7 in all 5 samples. This result indicates the possibility that Vβ7-bearing T cells in the middle ear are responding to a certain common antigen in some cases of OME.

KEY WORDS — αβ T cell, middle ear effusion, polymerase chain reaction, otitis media with effusion, superantigen, T cell receptor.

INTRODUCTION

Otitis media with effusion (OME) is one of the most prevalent middle ear diseases, characterized by the retention of effusions in the middle ear cavity.[1] The pathogenesis of OME likely involves the chronic accumulation of inflammatory and immune effector cells in the middle ear cavity. Cytologic studies of the middle ear effusions (MEEs) from patients with OME demonstrate the accumulation of significant numbers of lymphocytes.[2,3] Another recent piece of evidence suggests that in OME, an activated local immune system is present in the middle ear and the effusion is due to a local reaction.[4,5] In addition to that, there is an increasing number of reports that various kinds of cytokines were detected in MEEs.[6-10] Many of the cytokines are produced by T lymphocytes as a result of their interaction with antigen-presenting cells. The mechanisms by which T lymphocytes recognize and respond to antigen are of great importance in understanding OME and in the development of therapeutic interventions.

During the past few years, considerable progress has been made in our understanding of the molecular events involved in T cell recognition and in the processes involved in initiating the T cell response to foreign antigens. A large number of cell surface molecules on the T cell have been implicated in the recognition events that result in the initiation of a T cell response. However, of primary importance is the T cell antigen receptor (TCR), because it must be involved in the regulation of all antigen-specific T cell responses.[11] The antigen receptor (TCR) on most T cells is a heterodimer made up of α and β chains. The ligands for αβ TCRs are combinations of antigen-derived peptides, bound to major histocompatibility complex proteins. The wide diversity of T cell receptors is generated primarily through rearrangement of multiple V (variable), D (diversity), J (joining), and C (constant) gene segments at the TCR α and β gene loci during embryonic development, and through the random addition of nucleotides that are not part of genomic sequences to N regions, at V-D, D-J, and V-J junctions.[11] Recent studies suggest that important insights into the pathogenesis and therapy of disease may be obtained by the identification of particular TCR V region segments that are preferentially used in responses to defined antigens and in autoimmunity. For example, Vβ2 and Vβ13 genes were predominantly expressed on the T cells around minor salivary glands in patients with Sjögren's syndrome, suggesting the possible role of T cells bearing these Vβ TCRs in triggering the autoimmunity of this disease.[12] Toxic shock syndrome may result from the polyclonal activation of T cells that express particular Vβ region (Vβ2)–derived products reactive with staphylococcal enterotoxins.[13] Likewise, analysis of the repertoire of TCRs expressed in a diseased tissue sample is of crucial importance in understanding how the immune system functions and what kind of antigens (superantigens or conventional antigens) are involved in the immune system.

Since the precise mechanism of development of OME is not fully understood, we thought that analysis of the TCR repertoire in MEE might elucidate a

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TABLE 1. SUBJECTS STUDIED

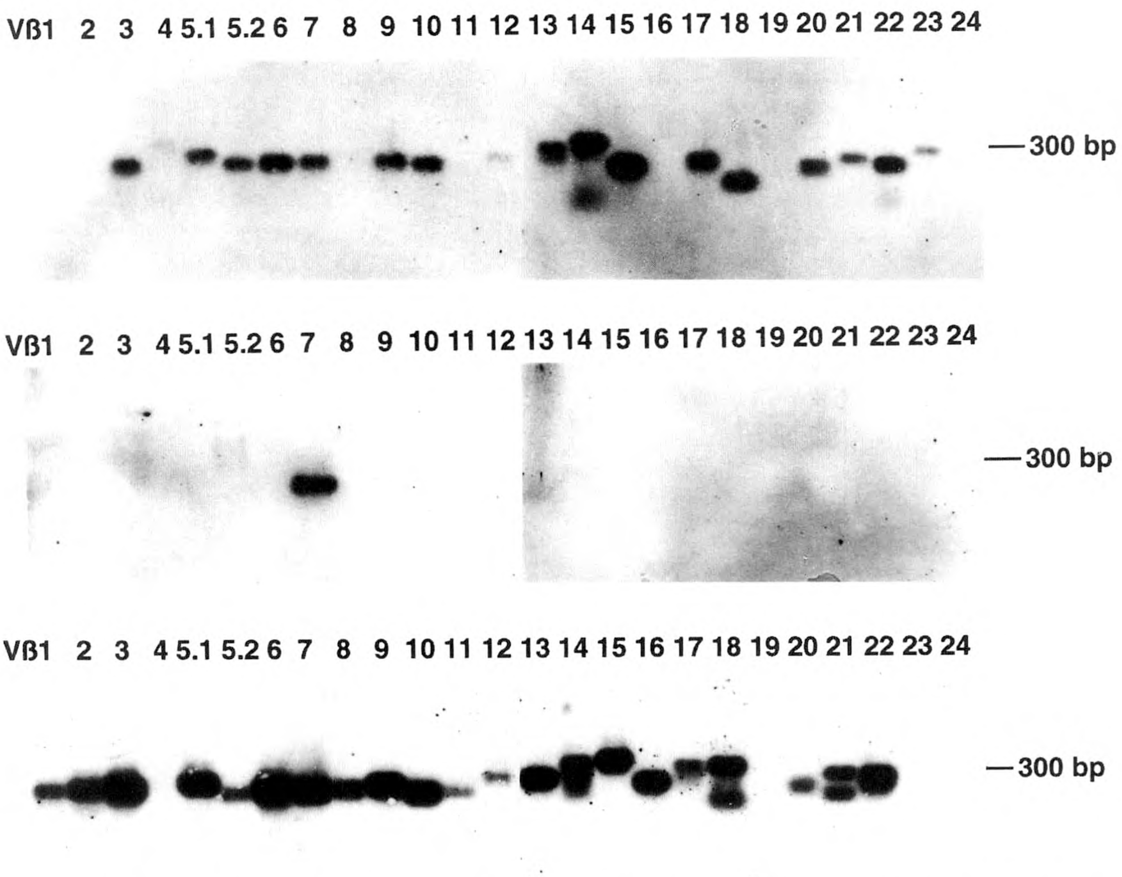
No.	*Age*	*Sex*	*Side*	*Effusions*	*Number of Vβ Families Utilized*
1	5	F	R	Mucoid	3
2	6	F	R	Serous	10
3	6	F	L	Serous	18
4	6	F	R	Mucoid	1
5	6	M	R	Serous	1
6	6	M	R	Mucoid	1
7	7	M	R	Mucoid	18
8	7	M	L	Serous	1
9	11	M	L	Mucoid	18
10	14	M	L	Mucoid	6
11	53	F	R	Serous	21
12	72	M	R	Serous	16
13	79	F	L	Serous	1

part of the immune response in OME. The present work was designed to analyze the TCR Vβ repertoire in MEEs by reverse transcription–polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Subjects. The OME was diagnosed by clinical symptoms, pneumatic otoscopy, pure tone audiometry, middle ear impedance measurement, and tympanometric patterns. The MEEs were aspirated from 10 ears of 9 children (5 to 14 years of age) and 3 ears of 3 adults (53 to 79 years of age) by means of the Juhn Tym-Tap (Xomed, Jacksonville, Fla) following myringotomy with aseptic precautions. The patient profiles are summarized in Table 1. None of the patients had any signs of acute infection, nor did they receive antibiotic therapy.

RNA and cDNA Preparation. Immediately after MEEs were aspirated, 200 μL of the denaturing solution (4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate, pH 7; 0.5% sarcosyl, 0.1 mol/L 2-mercaptoethanol) was added to MEEs. After vortexing the mixtures, we followed a single-step method of RNA isolation by Chomczynski and Sacchi.[14] Each RNA prepared as above was reverse-transcribed into complementary DNA (cDNA) with the GeneAmp


Vβ1 2 3 4 5.1 5.2 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
—300 bp
Vβ1 2 3 4 5.1 5.2 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
—300 bp
Vβ1 2 3 4 5.1 5.2 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
—300 bp

(Cases 9, 8, 11; top to bottom) Southern blot analysis of expression of Vβ segments in middle ear effusions from 3 patients with otitis media with effusion. For each sample, complementary DNA (cDNA) aliquots were subjected to polymerase chain reaction, using, in parallel reactions, each of 24 T cell receptor Vβ family-specific and common Cβ primers. Each lane contains cDNA samples assayed for amplification with indicated primers and hybridized with digoxigenin-labeled Cβ oligonucleotide.

TABLE 2. TCR Vβ EXPRESSION IN MIDDLE EAR EFFUSIONS

	Case No.														
Vβ Family	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*PBL**	*Total (% of 13)*
1	–	–	–	–	–	–	+	–	–	–	+	+	–	+	3 (23.1)
2	–	–	+	–	–	–	+	–	–	–	+	+	–	+	4 (30.8)
3	–	–	+	–	–	–	+	–	+	–	+	+	–	+	5 (38.5)
4	–	+	+	–	–	–	+	–	+	–	–	+	–	+	5 (38.5)
5.1	–	+	+	–	–	–	+	–	+	–	+	+	–	+	6 (46.2)
5.2	–	+	+	–	–	–	+	–	+	–	+	+	–	+	6 (46.2)
6	–	+	+	–	–	–	+	–	+	–	+	+	–	+	6 (46.2)
7	–	+	+	+	+	+	+	+	+	–	+	+	+	+	11 (84.6)
8	+	+	+	–	–	–	+	–	–	+	+	+	–	+	7 (53.8)
9	–	+	+	–	–	–	+	–	+	–	+	+	–	+	6 (46.2)
10	–	–	–	–	–	–	+	–	+	–	+	–	–	+	3 (23.1)
11	–	–	–	–	–	–	–	–	–	–	+	–	–	+	1 (7.7)
12	–	+	+	–	–	–	+	–	+	–	+	+	–	+	6 (46.2)
13	–	–	+	–	–	–	+	–	+	–	+	+	–	+	5 (38.5)
14	+	–	+	–	–	–	+	–	+	–	+	–	–	+	5 (38.5)
15	–	–	+	–	–	–	–	–	+	–	+	+	–	+	4 (30.8)
16	–	–	–	–	–	–	–	–	–	+	+	–	–	+	2 (15.4)
17	–	–	+	–	–	–	+	–	+	+	+	–	–	+	5 (38.5)
18	–	+	+	–	–	–	–	–	+	+	+	+	–	+	6 (46.2)
19	–	–	+	–	–	–	–	–	–	+	–	–	–	+	2 (15.4)
20	–	+	–	–	–	–	+	–	+	–	+	–	–	+	4 (30.8)
21	–	–	+	–	–	–	+	–	+	–	+	–	–	+	4 (30.8)
22	+	–	–	–	–	–	+	–	+	–	+	+	–	+	5 (38.5)
23	–	–	+	–	–	–	–	–	+	+	–	–	–	+	3 (23.1)
24	–	–	–	–	–	–	–	–	–	–	–	+	–	+	1 (7.7)

+ — presence of band, – — absence of band.

*As positive control, complementary DNAs prepared from human peripheral blood leukocyte (PBL) were similarly amplified.

RNA PCR Kit (Takara Biomedicals, Tokyo, Japan). Random hexamers were used as primers for generation of cDNA. After incubation at 42°C for 15 minutes, the samples were heated for 5 minutes at 99°C to terminate the reactions, and were stored at –20°C until used. One thirtieth of each cDNA sample was amplified by means of a Vβ-specific primer with a Cβ primer supplied by a T-cell receptor typing amplimer kit (Clontech, Palo Alto, Calif). This amplimer kit contains a panel of twenty-five 5'-primers for 24 known major Vβ families and a 3'-primer that anneals to the Cβ region. All the primers in the kit have been so designed that amplification occurs across exons (V, D, J, and C) separated by large intron sequences. Thus, amplification of genomic DNA is prevented. Expected lengths of PCR products range from 180 to 270 bp. The reaction mixture made at the manufacturer's suggestion was amplified for 35 cycles in a Perkin-Elmer/Cetus (Norwalk, Conn) thermal cycler. The amplification profile consisted of denaturation at 95°C for 1 minute, primer annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. Both negative and positive control experiments were performed with distilled water and human peripheral blood leukocyte cDNA as DNA templates, respectively.

Hybridization With End-Labeled Oligonucleotide Probes. In experiments designed to validate the PCR products, 20 picomoles of a Cβ probe internal to the amplified fragments were end-labeled with digoxigenin by means of the DIG oligonucleotide tailing kit (Boehringer Mannheim Yamanouchi, Nagoya, Japan). The PCR products were electrophoresed in 1.5% agarose gel and were blotted onto nylon membranes. The nylon membranes were prehybridized in 30 mL of buffer composed of 0.25% sodium dodecyl sulfate (SDS), 6× standard saline citrate (SSC), 2× Denhardt's reagent, and 100 µg/mL salmon sperm DNA at 43°C for 1 hour. Hybridization was conducted in the presence of the labeled oligonucleotide probe in the same solution as in prehybridization by incubation at 43°C for 2 hours. The blots were washed in 2× SSC, 0.05% SDS at 33°C for 60 minutes and at 53°C in the same solution for 3 minutes. Immunologic detection was performed as recommended by the manufacturer.

RESULTS

The Figure illustrates Southern hybridization of the PCR products in the three representative samples. Each Vβ-Cβ primer yielded a band of 180 to 270 bp, which is the expected length. No higher bands were amplified, indicating that no genomic DNAs were amplified. The number of Vβ families expressed in each sample is shown in Table 1. In most of the cases, more than one Vβ family has been amplified. In 5 of 13 samples, only 1 Vβ family was amplified. In the remaining 8 samples, the number of Vβ families found varied from 3 to 21. Table 2 shows which Vβ families were expressed in each sample. Vβ7 was the most frequently found, being expressed in 11 of 13 samples (84.6%). This was followed by Vβ8 (53.8%). Most strikingly, all 5 samples in which only 1 Vβ family was found expressed Vβ7.

Effusions were obtained from bilateral ears from 1 patient (cases 2 and 3). Although the Vβ families found in the 2 samples were not exactly the same, there was a significant overlap. As a positive control, we amplified human peripheral blood leukocyte cDNA, which gave bands in all the Vβ families. No bands were observed when distilled water containing no DNA templates was amplified.

DISCUSSION

The TCR Vβ repertoire of T cells in MEEs in OME patients was examined with the RT-PCR method. To our knowledge, this is the first report on the TCR repertoire in OME. Since the amounts of the starting mRNAs extracted from MEEs were too small to be quantitated, the limited number of Vβ families amplified by the method does not necessarily mean that the limited number of Vβ families is found in MEEs. However, we obtained evidence that Vβ7 was most frequently found, especially in the 5 effusions in which only 1 Vβ family was amplified. This result suggests that this TCR may respond to a major epitope of an antigen involved in the pathogenesis of OME. The TCR repertoire in the remaining 8 samples was heterogeneous. This heterogeneity could be explained by the heterogeneity of bacteria cultured from MEEs[15] or by different epitopes from a single bacterial strain produced, for example, by differing degrees of protease digestion. The heterogeneity might also result from different stages of an immune response.

Choi et al[13] reported that T cells expressing certain Vβ genes could be stimulated by some bacterial toxins, such as toxic shock syndrome toxin and staphylococcal enterotoxin, as a "superantigen." Whereas a conventional antigen complexed to major histocompatibility complex will react with antigen-specific T cells that represent a relatively small percentage of the T-cell pool ($1/10^4$ to $1/10^6$), superantigens stimulate a large proportion of T cells (1/20 to 1/4) that express the same TCR Vβ family structure irrespective of their antigen specificity.[16] For example, about 50% of the T cells in toxic shock syndrome toxin 1–stimulated T-cell blasts had Vβ2.[13] In this study, only Vβ7 was detected in 5 individuals, which could mean that most of the T cells present in the 5 middle ear effusions had Vβ7. This fact points to the involvement of a superantigen rather than a conventional antigen in the causation of OME in the 5 individuals.

Most of the superantigens known to date are bacterial toxins such as staphylococcal enterotoxins, toxic shock syndrome toxin 1, exfoliating toxins, streptococcal pyrogenic exotoxins, and *Mycoplasma arthritidis* supernatant.[16] Although these toxins are not reported to be present in MEEs, it is known that some bacterial antigens are retained in MEEs. Miller et al,[17] by dot-blot assay or latex agglutination, demonstrated the presence of *Streptococcus pneumoniae* and *Hemophilus influenzae* antigen in 21% and 26% of human MEE samples, respectively. Since none of the bacterial superantigens reported to date has specificity for Vβ7,[13,16] it is possible that an unknown bacterial superantigen is present in the MEEs. Another possibility is that a constituent of the middle ear is altered by inflammation and works as a superantigen. Recently, Conrad et al[18] reported that a pancreatic islet cell membrane-bound superantigen is involved in the causation of insulin-dependent diabetes mellitus, where a selective expansion of T cells having Vβ7 is observed. Preferential usage of Vβ7 genes is also reported in synovial fluid in rheumatoid arthritis.[19]

Previous reports have demonstrated the presence of viable bacteria in about half of OME cases. Treatment has been directed at eliminating these pathologic organisms, although resolution of the effusion does not always follow. There is a possibility that retained bacterial antigens or other antigens in MEE are responsible for activating T cells and cytokine productions, thus contributing to the persistence of middle ear inflammation and effusion. Elucidation of the TCR repertoire and the epitope will lead to a more rational approach to the treatment of OME. If the epitope is elucidated, the development of OME can be prevented by using peptide analogs of the pathogenic peptide or by vaccination with synthetic peptides derived from the Vβ sequence of the TCR expressed by the pathogenic T cells. Established active experimental allergic encephalomyelitis in mice has been treated successfully with monoclonal antibody reactive with its Vβ.[20]

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POSTURAL CHANGES IN RESPIRATORY AIRFLOW PRESSURE AND RESISTANCE IN NASAL, HYPOPHARYNGEAL, AND PHARYNGEAL AIRWAY IN NORMAL SUBJECTS

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We investigated the effect of posture on nasal and pharyngeal resistance in 12 healthy subjects studied during wakefulness. Airway pressure and airflow were measured with subjects seated and in dorsal and left lateral recumbency, during inspiration and expiration. We found that pharyngeal resistance was approximately four to six times lower than the nasal resistance. Only pharyngeal resistance was significantly increased upon assumption of a supine posture, from 0.02 ± 0.01 Pa/mL per second when seated to 0.06 ± 0.05 Pa/mL per second in dorsal recumbency and to 0.05 ± 0.04 Pa/mL per second in left lateral recumbency. Mean nasal and pharyngeal resistances doubled upon assumption of a supine posture, but this difference was not statistically significant. There was no significant difference in pharyngeal resistance beween inspiration and expiration. Finally, there was a strong linear relationship between pharyngeal pressure and pharyngeal resistance ($r = .98$, $p < .0001$). We concluded that in normal awake subjects 1) pharyngeal resistance increases with assumption of a supine posture, 2) the walls of the pharynx are not compliant enough to alter their resistance in response to inspiratory and expiratory pressure changes, and 3) it may be possible to infer pharyngeal resistance from measurements of pressure alone, without measurement of airflow.

KEY WORDS — hypopharyngeal resistance, nasal resistance, pharyngeal resistance.

INTRODUCTION

It is well accepted that patients with obstructive sleep apnea obstruct most frequently in the pharyngeal segment, which is usually less stable than the nasal, laryngeal, or tracheobronchial airway.[1-3] That the obstruction commonly occurs in the retropalatal region suggests that this airway segment has the highest compliance and therefore should exhibit large changes in resistance between inspiration and expiration. Furthermore, in many patients with sleep apnea, changes in sleep posture (from recumbent to semiupright) lead to significant reduction in the number of obstructive events; hence, postural changes may be associated with changes in upper airway resistance. Although the effect of posture on pharyngeal geometry and resistance has been described,[2,4-8] few, if any, studies report direct and simultaneous measurements of upper airway resistance for several segments of the upper airway. To tackle this problem, we first decided to study normal subjects during wakefulness, and measured airway resistance directly (by measuring airway pressures and respiratory flows) of the nasal (atmosphere to nasopharynx [A-N]), hypopharyngeal (atmosphere to hypopharynx [A-H]), and pharyngeal (nasopharynx to hypopharynx [N-H]) airway segments.

METHODS

We studied 12 normal subjects recruited among the laboratory personnel. All were men ranging in age between 33 and 75 years, non-snorers, on no medications, healthy, and without any symptoms that would suggest possible sleep-disordered breathing. All measurements were performed during wakefulness under three experimental conditions with subjects 1) seated, 2) during dorsal recumbency, and 3) during left lateral recumbency.

Pressures were measured at two points in the upper airway, nasopharynx and hypopharynx, with two small catheters (a nasal one and a hypopharyngeal one), usually used as infant feeding tubes. The nasal catheter's measuring port was at its tip, located 8 cm from the nares. The hypopharyngeal catheter was also passed through the nose into the esophagus. Its distal (lower) opening was closed with silicone, and a lateral opening 2 mm in diameter located 10 cm proximal to (above) the catheter's tip was used as the measuring port. The 10-cm blind end allowed for

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UPPER AIRWAY PRESSURES AND AIRFLOW WITH SUBJECTS SITTING AND RECUMBENT

	Seated	*Dorsal Recumbency*	*Lateral Recumbency*
Airflow	249 ± 89	208 ± 53	224 ± 59
ΔP A-N	32.6 ± 17.0	54.5 ± 33.4	49.4 ± 23.0
R A-N	0.15 ± 0.07	0.29 ± 0.18	0.24 ± 0.11
ΔP A-H	40.6 ± 18.5	68.2 ± 43.4	61.1 ± 24.1
R A-H	0.17 ± 0.10	0.32 ± 0.15	0.28 ± 0.12
ΔP N-H	4.0 ± 3.2	15.2 ± 10.3	12.8 ± 10.3
R N-H	0.02 ± 0.01	0.06 ± 0.05*	0.05 ± 0.04*

All pressures measured with respect to atmospheric pressure outside airway.

ΔP — differential pressure (pascals), R — resistance (pascals per milliliter per second), airflow — milliliters per second, A-N — atmosphere to nasopharynx, A-H — atmosphere to hypopharynx, N-H — nasopharynx to hypopharynx.

*Significantly ($p < .005$) different from value in seated position.

better stability of the catheter in the esophagus and avoided irritation of the hypopharynx during coughing and swallowing. The catheter dimensions and the pressure transducers used are identical to those reported previously.[9]

After ascertaining that the hypopharyngeal catheter had passed into the esophagus, we gradually withdrew it in 1 cm-increments until the lateral opening was in the hypopharyngeal airway, between the esophageal inlet and the midportion of the epiglottis. To verify the proper placement of the catheter, we performed several maneuvers. First, we injected 10 mL of air when the lateral port was still in the esophagus. This produced a characteristic uncomfortable feeling in the patient's chest. When the lateral port was above the esophagus, injection of air produced no discomfort and in fact the injected air could be heard from the patient's mouth. Finally, before the catheter was finally taped for recording, we ensured its proper position in the hypopharynx by indirect laryngoscopy or, if necessary, by flexible endoscopy.

Since both catheters were small, they were tolerated well by the subjects, and no topical anesthesia was required. Throughout the measurements the catheters were periodically flushed with air to avoid plugging of the ports by secretions. Identical methodology was used to measure differential pressures in all three airway segments (A-N, A-H, and N-H).

Respiratory airflow was measured exclusively during nasal breathing and was achieved by taping the mouth before starting the recordings. The method of measurement differed somewhat depending on whether the subject was seated or recumbent. With the subject in the seated position, we employed a head-out body plethysmograph[10] routinely used for clinical measurements of nasal resistance. With the subject in the dorsal position, we used a nasal mask (similar to the one used for administration of continuous positive airway pressure) equipped with a No. 400 mesh nylon laboratory filter at the airway port to serve as a pneumotachograph. This provided a low resistance and a linear airflow-pressure relationship between mask and atmosphere, and was more convenient than using a head-out body plethysmograph in the recumbent subjects. The equivalence of the two methods has been demonstrated previously over a wide range of nasal resistances.[10]

Pressure and airflow signals were sampled by an analog-to-digital converter and stored for subsequent analysis. They were also simultaneously displayed on a computer screen so that the measurements could be taken when the breathing signals were stable and free of artifact. In each subject we used pressure and airflow signals to obtain breath-by-breath nasal resistance over five breaths and recorded the corresponding pressure, airflow, and nasal resistance when the coefficient of variation for the five measurements was less than 5%. At least three sets of such measurements were obtained, and the average values of pressure, airflow, and nasal resistance were calculated and recorded for subsequent data analysis.[11]

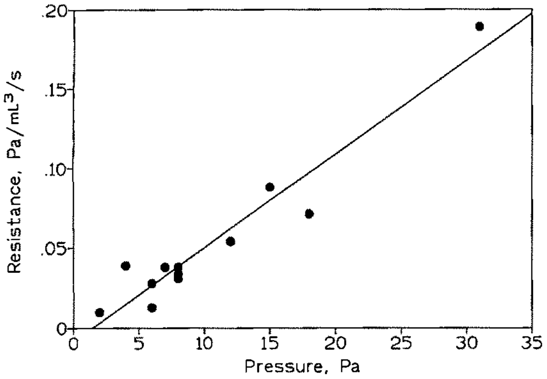
Analysis of variance with Tukey post-hoc tests was used to compare nasopharyngeal, hypopharyngeal, and pharyngeal pressures and resistances for all three postures.

RESULTS

The Table shows differential pressures and resistances in each of the segments in all three postures. Resistance in the pharynx was much lower than that in the A-N, averaging approximately 15% of the nasal resistance.

We found that all segmental upper airway resistances tended to be higher with subjects in the supine rather than in the seated position. This difference was statistically significant ($p < .005$) and most apparent for the pharynx, as the resistance almost quadrupled when the subjects assumed recumbency; the mean A-N and A-H airway resistances almost doubled, but the difference failed to reach statistical significance. There was no significant difference in pressures and resistances between dorsal and lateral recumbent positions for any of the airway segments.

Another feature that is evident from the Table is the significant variability of all upper airway resistances, in particular the pharyngeal resistance. Coefficients of variation (standard deviations divided by the mean)



Pharyngeal (nasopharynx to hypopharynx) pressure versus resistance for 12 subjects ($r = .96$, $p < .0001$).

ranged between 44% and 60% for nasal and hypopharyngeal resistance, and between 77% and 89% for pharyngeal resistance. No significant difference was found in nasal, hypopharyngeal, and pharyngeal resistances between inspiration and expiration.

The Figure shows the relationship between pharyngeal resistance and pharyngeal pressure, obtained from linear regression analysis. We note that the relationship is nearly linear and both variables are highly correlated ($r = .96$, $p < .0001$). This suggests that in future measurements it may be possible to infer resistance (which is a relatively complex measurement) from pressure (a relatively simple measurement), provided the flow conditions present during the current measurements are satisfied.

DISCUSSION

This study illustrates that upper airway resistance depends on a subject's posture, being higher in the supine than in the seated position; there was no significant difference in pharyngeal resistance between dorsal and lateral recumbency.

To arrive at this conclusion, we employed the most direct method of determining upper airway resistance: measurements of airflow and pressure along the upper airway. Although the methodology of such measurements is well established, the measurements themselves are not trivial and can be extremely variable both within and among subjects. Pharyngeal resistance is much more variable than resistances of the nasal, laryngeal, and tracheobronchial airways, which receive firmer skeletal support. Similar to previous investigators,[12-14] we demonstrated that resistance of the pharynx is lower than that of other segments.

There are several factors that may account for this variability, noted previously by ourselves[1] as well as by other investigators.[2,3,11,15] One factor is related to the insertion and manipulation of the pharyngeal catheter. We found it necessary to wait several minutes after catheter insertion before starting measurements, until stable breathing resumed, the amount of secretions was reduced, and the subject was comfortable. Another factor is the increased pharyngeal irritability that may accompany even a minor respiratory infection; this promotes instability, reduces patency, and increases pharyngeal secretions. None of our subjects had an obvious upper respiratory tract infection at the time of measurement, but we cannot rule out the existence of subclinical, inapparent pharyngeal irritability. Finally, the effect of head and neck position on pharyngeal patency must be considered.[16,17] Although we did not control the position of the head with cephalostats, we tried to maintain the same posture in all subjects by visual means, ensuring that they were comfortable and the pressure and airflow tracings were stable over many breaths in the seated and recumbent positions. We interpret our results as being illustrative of high individual variability in pharyngeal patency among different subjects. It is possible that the great variability of the measurements, coupled with the relatively few subjects examined here, accounted for the lack of postural differences in nasal and hypopharyngeal resistances. However, the fact that we found significant postural changes in pharyngeal resistance, despite similar interindividual variability, would mitigate against this possibility.

We, like Yildirim et al,[4] found that the pharyngeal resistance almost quadrupled upon the subject's assumption of a supine posture. This postural increase in upper airway resistance is consistent with many direct and indirect measurements of airway dimensions and resistance, whether measured by cephalometry,[4] acoustic reflections,[5,8] or airway pressures and airflows.[2] It is interesting that the only study that examined pharyngeal area in dorsal and lateral recumbency[8] failed to detect any difference between these two postures, but similar to the present study, found significant reduction in pharyngeal area between sitting and supine positions. However, the mechanism of postural dependence of airway resistance or dimensions remains obscure. Neck flexion, gravitational forces, and differential activation of upper airway muscles are all thought to play a role in modulating upper airway patency and resistance. Another possibility that may affect upper airway resistance is breathing route: oral versus nasal or oronasal. All of our subjects had their mouths taped and breathed exclusively through the nose, the most resistive route, which augments the activity of the genioglossus muscle.[18] However, the recent study by Douglas et al[19] indicates that postural effects on genioglossal activity are independent of the effect of breathing route.

Ultimately, the importance of studying postural changes in upper airway resistance is linked to better understanding of the pathophysiology of obstructive sleep apnea. Marked increase in the pharyngeal resistance in dorsal recumbency is consistent with the common observation that patients with sleep apnea have more apneas when lying on their back. In fact, many such patients prefer to sleep in a semirecumbent position in order to reduce the number of obstructive events. It may be hypothesized that in patients with sleep apnea, assumption of a supine posture and increased negative pressure during inspiration would tend to collapse the pharynx and lead to airway obstruction. However, in our normal subjects we found no evidence of pharyngeal collapsibility, with pharyngeal resistances being not significantly different during inspiration and expiration. This may not be the case in patients with sleep apnea.

Finally, at least in normal subjects it appears that the relationship between pressure and airflow is linear; if this is the case in patients with sleep apnea, upper airway resistance may be deduced from pressure measurements alone, without the necessity of measuring airflow.

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EFFECT OF OXYMETAZOLINE NOSE DROPS ON ACUTE SINUSITIS IN THE RABBIT

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This investigation was designed to evaluate the effect of the common topical nasal decongestant oxymetazoline hydrochloride on the early local tissue defense in an experimental bacterial infection. For that purpose, a bilateral infection of the maxillary sinus was induced in 14 rabbits. Nose drops (oxymetazoline) were instilled in one nasal cavity, and placebo in the other. After 48 hours, the degree of infection was judged by using a semiquantitative inflammatory score to evaluate histologic preparations of the maxillary sinuses. On the oxymetazoline-treated side, we found a significantly higher degree of inflammation. We conclude that oxymetazoline nose drops, commonly used in acute rhinitis and sinusitis, interfere with the normal defense mechanisms during bacterially induced sinusitis, possibly by a decrease in mucosal blood flow.

KEY WORDS — bacterial infection, experimental infection, oxymetazoline, sinusitis, topical nasal decongestants.

INTRODUCTION

Topical nasal decongestants (nose drops) fall into two categories — namely, the sympathomimetic amines (eg, phenylephrine hydrochloride) and the imidazoline derivatives (eg, oxymetazoline hydrochloride, xylometazoline hydrochloride). The congestion of the nasal mucosa in acute rhinitis is due to vasodilation of the sinusoids and to interstitial edema. Nose drops act by constricting the sinusoids in the nasal mucosa. The onset of action is rapid and the effect is long-lasting.[1] These positive effects have resulted in widespread use of these drugs, especially as they are often sold over the counter. The indication for nose drops is the common cold, but many people continue to use nose drops for months and even years, and rebound congestion then develops.[2-6]

Imidazoline derivatives are selective $\alpha 2$-adrenoceptor agonists, while sympathomimetic amines act on $\alpha 1$-adrenoceptors.[7] These differences in mode of action may be important, since the sinusoids in the human nasal mucosa are regulated by $\alpha 1$- as well as $\alpha 2$-adrenoceptors, while the nasal mucosal blood flow is not significantly affected by $\alpha 1$-adrenoceptor agonists in humans.[8,9] Treatment with nasal decongestants of the imidazoline type leads to a decrease in blood flow, along with their desirable decongestive effect, and the duration of these effects is about 6 hours.[1,10] In clinical doses the reduction in the nasal mucosal blood flow of oxymetazoline nose drops is about 50% in healthy subjects and about 60% in patients with the common cold.[10]

The increase in blood flow is a cardinal sign in infectious conditions. Drugs interfering with blood flow may modify the local defense reaction and may counteract an adequate inflammatory response. There are few basic studies regarding the effects of topical nasal decongestants on parameters other than decongestion. This study was conducted to investigate the early effect of oxymetazoline nose drops on an experimentally induced bacterial sinusitis.

MATERIALS AND METHODS

Bilateral experimental sinusitis was induced in 14 New Zealand White rabbits weighing approximately 2 kg. Oxymetazoline nose drops were instilled in the nasal cavity in one side, and placebo in the other side, three times daily for 2 days. Thereafter, the sinus mucosa was examined with a light microscope and the two sides were compared regarding the degree of inflammation. On the first day, when general anesthesia was given (Saffan, Cooper-Pittman-Moor, England, 6 to 9 mg/kg), the superior part of the maxillary sinuses of the rabbits was exposed surgically and a hole was drilled through the bony naso-

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From the Department of Applied Mechanics and Engineering Sciences, University of California–San Diego, San Diego, California. This investigation was supported by Public Health Service Fogarty International Research Fellowship #1 FO5 TWO4483-01, the Swedish Medical Research Council (MFR 9379, 9283, and 00749), the Swedish Society of Medicine, Nanna and Albert Skantze's Memorial Foundation, Tore Nilsson's Foundation for Medical Research, the Skaraborg County Council, and US Public Health Service HL 17421. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Use and Care of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California–San Diego and Pharmacia Experimental Medicine.

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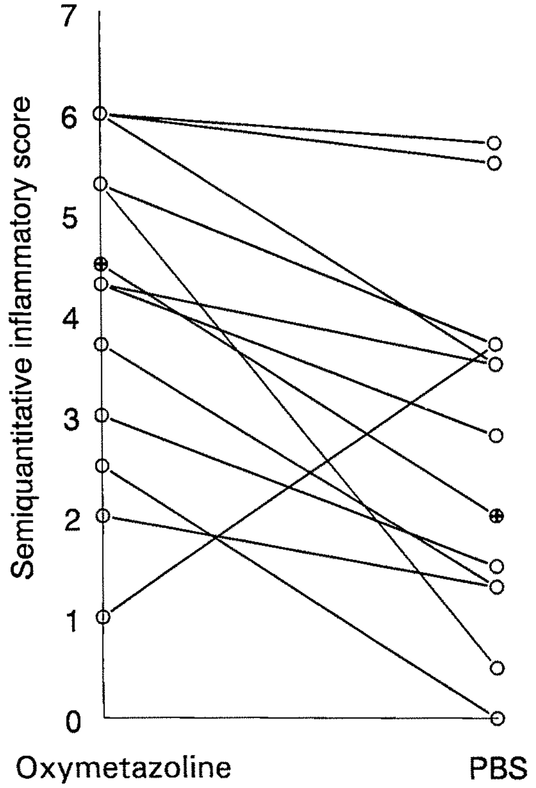

Fig 1. Degree of inflammation in maxillary sinuses of rabbit after infection was induced bilaterally. One side was treated with oxymetazoline nose drops, and other with placebo (phosphate-buffered saline [PBS]). Scores of two animals coinciding with each other are specially marked.

dorsal wall of the sinus on both sides. The skin incisions were then sutured.

A well-established method for inducing experimental sinusitis was used.[11-13] On the second day, 10^8 colony-forming units of *Streptococcus pneumoniae* in 1 mL saline with a 2% agarose solution was deposited bilaterally in the maxillary sinus with a sterile syringe through the skin, the bone hole, and the intact sinus mucosa. Thereafter, 0.1 mL of oxymetazoline nose drops (0.5 mg/mL) was deposited in one nasal cavity with a small nasal catheter, and phosphate-buffered saline (PBS) nose drops with the same pH were deposited on the other side as a blind control. (The vehicle of the oxymetazoline solution was PBS.) This procedure was repeated every eighth hour for 48 hours. The catheter was designed to deposit the nose drops in the middle meatus, in the vicinity of the ostium.

The animal was then painlessly sacrificed and the nose and the sinus cavity system were removed. Fixation was performed with 6% glutaraldehyde, 2% paraformaldehyde in phosphate buffer, and the specimen was decalcified. The whole-mounted nose-sinus complex was serially sectioned. In every rabbit, four levels were evaluated. On each level three slides were stained with hematoxylin-eosin, Alcian blue–periodic acid–Schiff (pH 2.5), and May-Grünwald–Giemsa stain, respectively. The specimens were examined with whole-mount light microscopy. Levels anterior and posterior to the ostium of the maxillary sinus were selected for assessment. The histopathologic examination was performed by a pathologist, who assessed the inflammation in a blind procedure. Semiquantified grading was performed regarding 1) leukocyte infiltration, 2) mucosal edema, and 3) epithelial metaplasia. Each parameter was judged with a seven-grade scale ranging from 0 (no inflammation) to 7 (severe inflammation). The means of all parameters graded on the treated and the control sides in the same animal were statistically compared.

RESULTS

The histopathologic examination showed a pronounced tissue cellular reaction in the sinus mucosa. In general, the mucosal inflammatory response was more pronounced on the oxymetazoline-treated side. The sinus cavities showed a more marked accumulation of inflammatory cells, with bacteria and secretions, as compared to the sinus mucosa.

The mean inflammatory score in the sinus on the same side as that treated with oxymetazoline in the nasal cavity was 4.2 ± 0.4 (mean ± SEM), and on the contralateral, PBS-treated side it was 2.6 ± 0.5 (Fig 1). The difference was statistically significant ($p < .01$, paired *t*-test). Figure 2 illustrates the histopathologic findings.

DISCUSSION

Previous investigations have shown that topical oxymetazoline reduces the blood flow in the nasal mucosa in a dose-dependent manner in the rabbit.[14,15] Furthermore, the instillation of oxymetazoline nose drops in the nasal cavity induces a decrease in the mucosal blood flow of the maxillary sinus, indicating that the main blood supply to the sinus cavity passes through the blood vessels of the ostium.[14] To mimic the effect in humans,[10] the dose of oxymetazoline in this study was chosen to produce a reduction in the sinus mucosal blood flow of around 50%.

In addition to a 50% reduction in blood flow in the mucosa, a phenomenon called vasomotion occurs in the rabbit.[14,15] Vasomotion consists of periodic oscillations of blood in the microcirculation, and it is presumed to be a mechanism that optimizes tissue perfusion during low-flow conditions. Thus, vasomotion develops when the reduction in blood flow is about to become harmful to the tissue.

In a previous investigation, oxymetazoline nose drops were found to cause inhibition of leukocyte

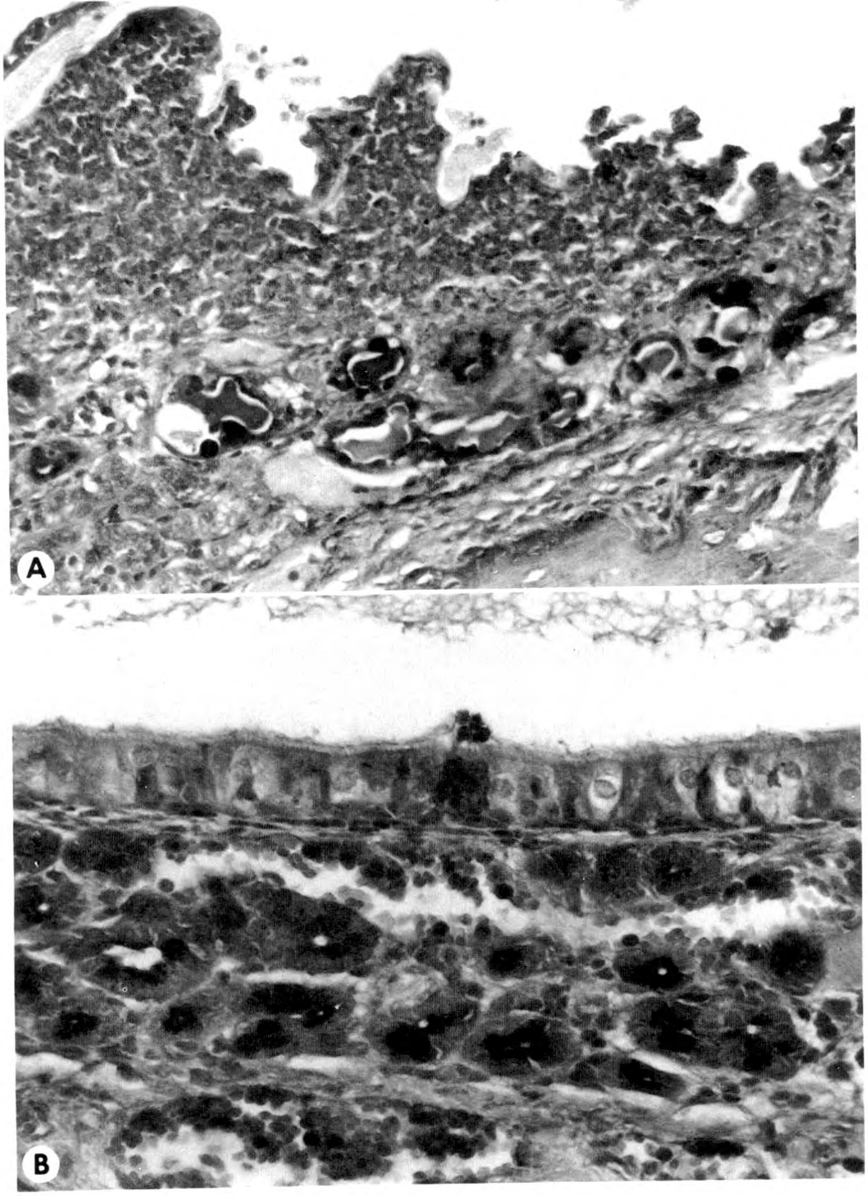

Fig 2. Histopathologic findings in sinus mucosa in rabbit (Alcian blue–periodic acid–Schiff, original ×125). A) Oxymetazoline-treated side, showing marked inflammatory reaction with epithelial metaplasia and desquamation, as well as gland involution. B) Phosphate-buffered saline–treated side, displaying leukocyte infiltration and edema, but with intact epithelium.

migration during the first 2 hours of a provoked inflammatory reaction.[16] This inhibition may be due to the reduction in blood flow, but may also be caused by an effect on the endothelium in the microvascular bed. This finding is supported by another study, in which topically applied oxymetazoline reduced vascular permeability to albumin after provocation with leukotriene B4 in the nasal mucosa.[17]

The cellular response in the nasal mucosa and in nasal secretions in individuals with common colds shows that most of the lymphocytes are found in the secretions rather than in the mucosa.[18,19] A similar distribution of inflammatory cells between the mucosa and the secretions of the maxillary cavity was also seen in the present study.

In this study, oxymetazoline apparently interfered with the inflammation. The result was taken to mean that without oxymetazoline, the inflammation rapidly and more effectively was combated within 2 days. Another explanation, or partial explanation, might be that oxymetazoline inhibits the defense against microbes and that therefore a more marked inflammation developed. Since we do not know the individual bacterial counts in the sinuses or the consequences of this initially increased inflammation on the resolution of the sinusitis, the increased inflammation found in this study may be of advantage or disadvantage for the host. We found a more pronounced inflammation on the side treated with oxymetazoline nose drops than on the placebo-treated side. The interference of oxymetazoline nose drops with healing of the experimentally induced bacterial sinusitis could be due to a reduced local metabolism, in combination with a decrease in vascular permeability and effects on glandular activity. Another potential but less likely explanation for the increased inflammation after oxymetazoline treatment could be an adverse effect on mucociliary transport. The combination of a low oxygen content in the sinus cavity and a low oxygen content of the maxillary mucosa could have impaired the mucociliary clearance and thereby the local defense.

This study indicates that oxymetazoline nose drops have other and more important effects than decongestion. Although the results from these experimental studies may not be directly related to the human clinical situation, the extensive use of topically administered decongestive nose drops in harmless upper respiratory tract infections requires that these effects be evaluated, and further studies are therefore necessary.

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SCHNEIDERIAN-TYPE MUCOSAL PAPILLOMAS OF THE MIDDLE EAR AND MASTOID

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Five cases of schneiderian-type mucosal papillomas arising in the middle ear space are reported. The patients were all women, ranging in age from 19 to 57 years (median, 31 years). Clinical complaints — unilateral conductive hearing loss, pain, or otorrhea — ranged from those lasting several months to recurrent problems spanning 20 years. All of the patients had a history of chronic otitis media predating the development of the papillomas; none of the patients had a history of sinonasal or nasopharyngeal schneiderian-type papillomas. Clinically, three patients had intact tympanic membranes, while the other two patients had perforated tympanic membranes through which a bulging polypoid mass was identified. Radiographic studies showed opacification of the middle ear space without evidence of osseous destruction. The intraoperative findings were of polypoid lesions filling the middle ear space, including involvement of the eustachian tube orifice. Histologically, the tumors were identical to sinonasal schneiderian papillomas. Immunohistochemical evaluation for human papillomavirus was negative. Surgical excision is the treatment of choice. In four of the patients, recurrent tumor was identified, necessitating additional surgery. In only one patient did the initial surgery result in complete ablation of the tumor. All patients are alive and free of recurrent disease over periods ranging from 6 months to 120 months (median, 84 months).

KEY WORDS — human papillomavirus, mastoid, middle ear, schneiderian papilloma.

INTRODUCTION

Schneiderian papillomas represent a unique group of benign neoplasms arising within the mucosal surfaces of the sinonasal tract. These neoplasms are readily identified by their histomorphologic features and are characterized by their tendency to recur if inadequately excised. Infrequently, lesions with similar histologic and biologic characteristics may arise outside the embryological and anatomic confines of the sinonasal tract. Extra–sinonasal tract sites in which schneiderian-type papillomas have been reported include the pharynx,[1-3] lacrimal sac,[4] and middle ear space.[5-7] In these locations, the characteristic histologic features may not be fully appreciated in terms of their correlative biologic potential. As such, a specific diagnosis of schneiderian-type papilloma may not be made, and appropriate management may not be initiated. Similar to the papillomas of the sinonasal tract, the extra–sinonasal tract schneiderian papillomas may recur following inadequate excision, increasing patient morbidity. It is due to the rarity of these tumors in the middle ear and the potential for misdiagnosis and mismanagement that this study was undertaken. Also included is a discussion of their possible origin in the middle ear.

MATERIALS AND METHODS

A search of the Otolaryngic Tumor Registry at the Armed Forces Institute of Pathology revealed five cases coded as epidermoid papillomas of the middle ear space. One of these cases has been previously reported.[7] Hematoxylin and eosin–stained microslides, available in all cases, were reviewed. Inclusion in this study required 1) the histopathologic hallmarks identified in this group of lesions (see below) and 2) primary involvement of the middle ear space without evidence of a prior papilloma in the sinonasal tract or nasopharynx. Medical records, including the clinical history, treatment, and follow-up, were reviewed in each case.

Special histochemical stains for epithelial mucin using Mayer's mucicarmine and periodic acid–Schiff with and without diastase digestion were performed. Paraffin blocks were available from four of the cases, from which 5-μm sections were prepared for immunophenotypic analysis according to the standardized avidin-biotin method of Hsu et al.[8] The basic commercially prepared antibody panel for each case included cytokeratin cocktail (AE1/AE3) (mouse monoclonal, 1:200 dilution, Dako, Carpinteria, Calif) and chromogranin (mouse monoclonal, 1:1,600 dilution, Boehringer Mannheim, Indianapolis, Ind). Of the above antibodies, cytokeratin required predigestion. Cytokeratin predigestion was for 3 minutes with 0.05% Protease VIII (Sigma Chemical Co, St Louis, Mo) in 0.1 mol/L phosphate buffer at a pH of 7.6 at 37°C. In situ hybridization reactions were done on paraffin-embedded tissue sections. Slides were deparaffinized in xylene and then treated sequentially with 5 mL 30% hydrogen peroxide per 250 mL,

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0.05 mol/L of phosphate-buffered saline (PBS), and 250 μg/mL proteinase K (Boehringer Mannheim) for 10 minutes at 37°C, then PBS-washed. Slides were dried on a warming tray at 37°C. The human papillomavirus (HPV) DNA probes (Enzo, Farmingdale, NY) were added to the sections, which were then denatured on a heat block at 100°C for 10 minutes. Hybridization was performed at 37°C for 2.5 hours. Detection of hybridization DNA was accomplished by sequential incubation with mouse antibiotin (1: 2,000 dilution; Dako), biotinylated horse anti mouse IgG (1:400; Vector, Burlingame, Calif), and avidin-biotin-peroxidase complex (Vector). Washes between all steps were in PBS. The last wash was followed by the addition of a substrate consisting of a diaminobenzidine (DAB) working solution of 42 mg DAB to 300 mL PBS plus 0.4 mL of 30% hydrogen peroxide, which was added for 8 minutes, then washed with distilled water and counterstained with Mayer's hematoxylin for light microscopic evaluation. Positive and negative controls were used throughout.

RESULTS

Clinical Features. The clinical findings are detailed in the Table. In brief, all the patients were female, ranging in age from 19 to 57 years (median, 31 years). Clinical complaints included unilateral conductive hearing loss in three patients (cases 1, 4, and 5) and otorrhea in one patient (case 2). Otalgia was also noted in association with these complaints. The remaining patient (case 3) had congenital abnormalities, including a cleft palate, and bilateral atelectatic middle ear disease, including nonfunctioning eustachian tubes. She presented with (left-sided) suppurative chronic otitis media and was found to have aural "polyps." All complaints related to the left ear; the right ear in all of the patients was intact, without evidence of tumor. All of the patients had a long-standing history of otitis media spanning months to years prior to the identification of their tumors. Treatment for the otitis media included antibiotic therapy and/or myringotomy and ear tube placement. The appearance of the tympanic membrane varied from case to case, including membranes that were intact, without significant changes, to membranes that were retracted, flat, and dull in appearance, to perforated membranes from which a polypoid mass was seen to extrude. Radiographic studies, including computed tomography (CT) and magnetic resonance imaging, showed opacification of the middle ear space, without evidence of an aggressive process (ie, presence of bone destruction). Failure of the otitis media to resolve prompted exploratory tympanotomy, with the identification of polypoid mass lesions in the left middle ear space. There was no history in any of the patients of a prior middle ear neoplasm, nor was there evidence of a previous or concurrent sinonasal tract or nasopharyngeal papillomatous proliferation.

Treatment and Follow-up. The initial treatment in three of the patients (cases 1, 4, and 5) was myringotomy with simple surgical excision, and in two of the patients (cases 2 and 3) a tympanomastoidectomy was performed. In the three patients with myringotomy and simple excision and in one of the patients initially treated by tympanomastoidectomy (case 2), the tumor persisted or recurred. All patients experienced multiple recurrences (≥2) over periods ranging from 2 to 15 years following the initial surgery. Four of the patients underwent one or more conservative surgical procedures (cases 1, 2, and 4) or modified radical tympanomastoidectomy (case 5) to treat the recurrent tumor. Ultimately, these four patients required a radical tympanomastoidectomy in order to eradicate the tumor. Following this definitive surgical procedure, all of the patients have been disease-free, at 6, 60, 84, and 144 months. The remaining patient (case 3) underwent a tympanomastoidectomy as the initial surgical modality. For 120 months following this procedure, the patient has been disease-free. Follow-up has been accomplished by periodic radiographic (CT scan) evaluation.

Pathology. The intraoperative gross description of the tumor(s) varied and included a cauliflower-appearing mass to numerous grapelike or polypoid lesions filling the middle ear. These lesions were described as yellow-tan to tan-white and soft to rubbery, and measured from 0.2 cm to 0.7 cm in greatest diameter.

The histologic appearance in all of the cases was that of a polypoid epithelial cell neoplasm. The predominant growth pattern was exophytic, composed of papillary fronds with a fibrovascular core (Fig 1). In addition, an endophytic or inverted growth pattern was also identified (Fig 2). The epithelial component was composed of multilayered epidermoid and/or ciliated columnar cells showing cellular uniformity, cell maturation, and retention of cell polarity. The epidermoid-appearing cells had round to oval nuclei and a glassy-appearing cytoplasm (Fig 3). In areas, intercellular bridges could be identified. The columnar cells were characterized by the presence of cilia along the cell surface and by the presence of a granular eosinophilic-appearing cytoplasm (Fig 4). These different cell types were seen as isolated phenomena or were intimately admixed within the same case (Fig 5). Mitotic figures were seen, but were limited to areas near the basement membrane. Surface keratinization was not identified.

Mucocytes characterized by round cells with abun-

CLINICOPATHOLOGIC FEATURES OF MIDDLE EAR SCHNEIDERIAN PAPILLOMAS

Case	*Age*	*Sex*	*Year of Initial Presentation*	*Clinical*	*Pathologic Findings*	*Treatment*	*Follow-up From Most Recent Surgery*
1	31	F	1975	L conductive hearing loss and otalgia; history of COM; intact TM; numerous polypoid, grapelike lesions filling ME mass and creeping into eustachian tube; clinically felt to represent ME adenoma	Epidermoid papilloma with features of both inverted and cylindrical cell papilloma	Myringotomy with simple surgical excision; tumor recurred 7 times over 15 years, each time treated by myringotomy with simple surgical excision but ultimately necessitating radical mastoidectomy; more recent recurrent tumors noted to originate at orifice of eustachian tube	ANED at 60 months
2	56	F	1981	L otorrhea; polypoid mass protruding from perforated TM; ET identified firm cauliflowerlike mass in ME; history of refractory COM treated with antibiotics, and myringotomy and ear tube placement	Epidermoid papilloma with exophytic and endophytic growth	Tympanomastoidectomy; tumor recurred twice over 2 years, ultimately necessitating radical mastoidectomy	ANED at 144 months
3	19	F	1985	History of cleft palate and bilateral atelectatic ME disease with nonfunctioning eustachian tube; refractory L suppurative COM with otorrhea for 2 ears; intact TM; ET identified polypoid lesions in left ME felt to represent aural polyps	Epidermoid papilloma with features of cylindrical cell papilloma	Tympanomastoidectomy	ANED at 120 months
4	57	F	1982	L conductive hearing loss; long history of COM and otalgia for 13 years; intact TM; ET identified polypoid lesions in left ME felt to represent ME adenoma	Epidermoid papilloma with features of cylindrical cell papilloma	Myringotomy with simple surgical excision; tumor recurred twice over 6 years, treated by myringotomy and simple excision but ultimately necessitating radical mastoidectomy	ANED at 84 months
5*	19	F	1990	L conductive hearing loss for 1 year and progressive otalgia prompting medical attention; perforated TM from which flesh-colored mass was seen; at surgery, polypoid mass filling ME space was found encroaching on but not entering eustachian tube; history at age 6 of COM treated by bilateral myringotomy and placement of ear tubes	Epidermoid papilloma with features of cylindrical cell papilloma	Myringotomy with simple surgical excision; tumor recurred twice over 3-year period; initial recurrence was treated by modified radical tympanomastoidectomy; second recurrence was treated by radical tympanomastoidectomy; at each recurrence, tumor was always found at opening of eustachian tube	ANED at 6 months

COM — conductive hearing loss, TM — tympanic membrane, ME — middle ear, ET — exploratory tympanotomy, ANED — alive with no evidence of disease (recurrence or persistence).

*Reported by Roberts et al.[7]

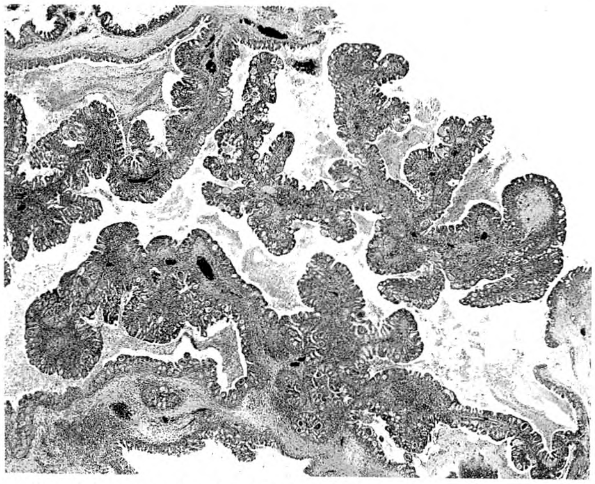

Fig 1. Exophytic schneiderian-type papilloma of middle ear with numerous papillae containing fibrovascular core. This "grapelike" tumor filled middle ear space.

dant foamy to basophilic-appearing cytoplasm and an eccentrically located compressed nucleus, and intraepithelial cystic spaces filled with inflammatory cells or mucinous material, were seen within the surface epithelial component (Figs 3 and 4). In addition, an acute and chronic inflammatory cell infiltrate was readily identified, permeating throughout the epithelial layer. Submucosal cysts were not identified in any of the cases. The nonneoplastic tissue showed changes of chronic otitis media, including chronic inflammation, fibrosis, glandular metaplasia, and cholesterol granulomas. These changes varied from case to case.

All of the recurrent tumors were histologically identical to the primary neoplasm. There was no histologic evidence in any of the cases of epithelial dysplasia or transformation to a malignant epithelial neoplasm. Only one case showed a separate pathologic process in addition to the middle ear schneiderian papilloma. In case 1, a cholesteatoma was identified, characterized by the presence of a keratinizing squamous epithelium with subjacent granulation tissue.

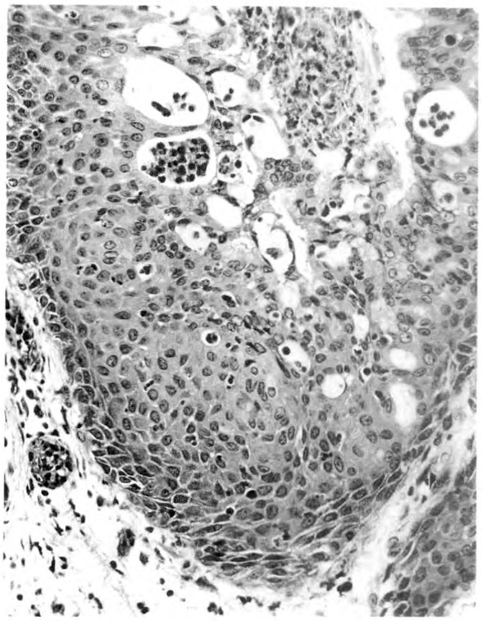

Fig 3. Epithelium of inverted papilloma is multilayered, composed of benign epidermoid cells. Cystic spaces filled with mucinous material and/or inflammatory cells and scattered mucocytes are seen.

Histochemistry and Immunohistochemistry. Stains for epithelial mucin showed both intracytoplasmic and extracellular mucicarminophilic and diastase-resistant, periodic acid–Schiff–positive staining. The positive staining was identified within the cytoplasm of the mucocytes, as well as within the intraepithelial cystic structures. Diffuse cytokeratin immunoreactivity was identified within the epithelial neoplastic cells. There was no immunoreactivity with chromogranin. Immunohistochemical evaluation for HPV particles (6/11 and 16/18) failed to demonstrate reac-

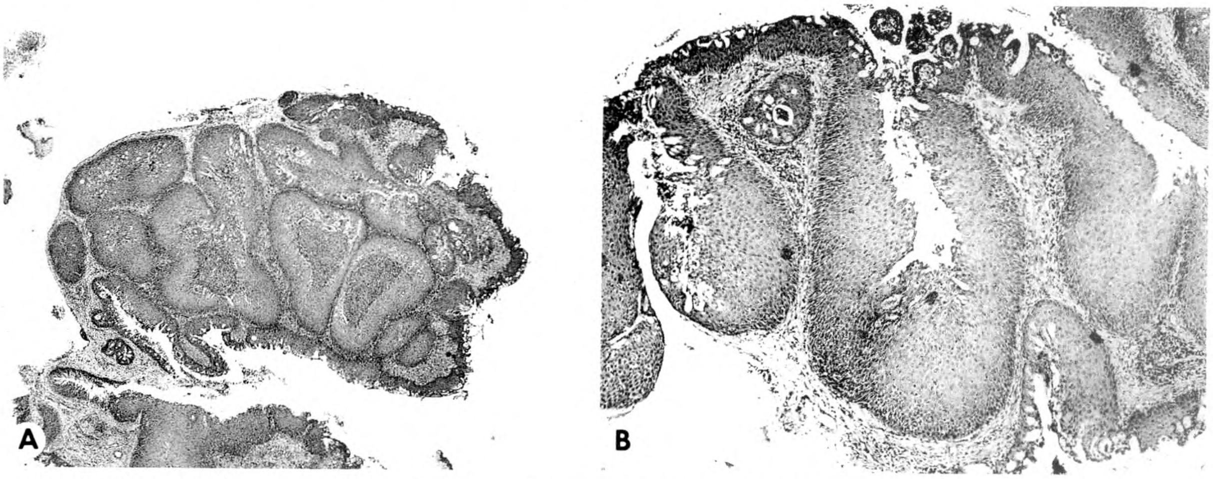

Fig 2. A) Schneiderian-type papilloma of middle ear in which epithelial component has endophytic or inverted growth. B) Higher magnification, showing characteristic downward growth of inverted papilloma.

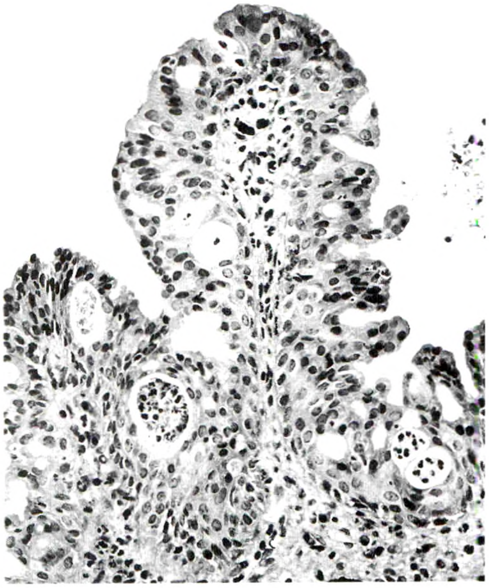

Fig 4. Papillary frond with thin fibrovascular core is seen in this middle ear cylindrical cell papilloma. In contrast to inverted papilloma, epithelium of cylindrical cell papilloma is characterized by ciliated columnar cells with granular eosinophilic cytoplasm. Cystic spaces filled with mucinous material and/or inflammatory cells are seen.

tivity.

DISCUSSION

The schneiderian papillomas represent a well-recognized group of benign sinonasal tract epithelial neoplasms with characteristic clinical and pathologic features. Among the different morphologic types of schneiderian papillomas are the inverted (endophytic), cylindrical, and fungiform (exophytic) types.[1,9] A diagnosis of schneiderian-type papilloma is indicative of a benign epithelial tumor that has the capability to recur if incompletely excised and in rare instances may undergo malignant transformation to a (nonkeratinizing) carcinoma. Armed with this information, the surgeon makes all attempts to visualize the entire extent of the tumor and to surgically eradicate the neoplasm in order to prevent recurrent tumor. The pathogenesis for schneiderian papillomas remains speculative. Etiologic considerations include neoplastic, viral, inflammation, allergy, and environmental exposure.[9] Human papillomavirus types 6/11 have been identified in association with sinonasal tract schneiderian papillomas.[10,11] Although no cause-and-effect relationship has been definitively proposed between the presence of HPV and the development of a schneiderian papilloma, this possibility must be strongly considered. Whether the mere presence of HPV can itself induce the development of a schneiderian papilloma remains to be proven. Most likely, the development of a schneiderian papilloma is not the result of a single inciting stimulus, but relates to many factors.

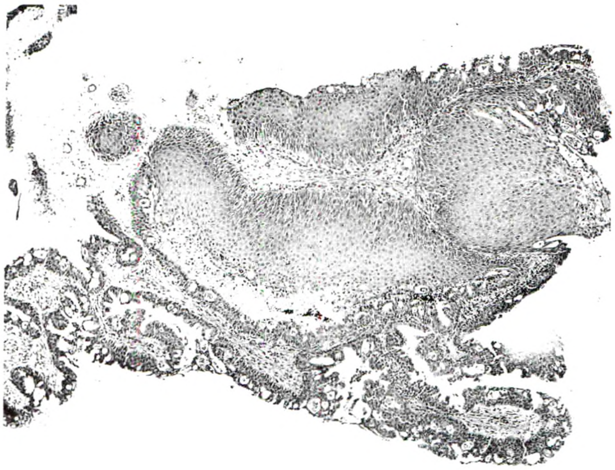

Fig 5. Middle ear schneiderian-type papilloma composed of admixture of inverted and exophytic (cylindrical cell) growth patterns.

Although uncommon, schneiderian-type papillomas can occur outside the sinonasal tract. “Ectopic” sites of involvement include the nasopharynx,[2,3] lacrimal sac,[4] and middle ear–mastoid region.[5-7] The schneiderian papillomas of the lacrimal sac should not be considered ectopic, as the nasolacrimal duct, which expands to form the lacrimal sacs of the eyes, develops from the same ectoderm that forms the schneiderian mucosa (the nasolacrimal groove).[12] To date, there are three previously reported cases of schneiderian-type papillomas arising in the middle ear or mastoid region.[5-7] One of these reported cases[7] is case 5 in this study. Two of these cases occurred in women and one in a man, with ages of 19, 55, and 77 years. The clinical complaints included conductive hearing loss, otalgia, and otorrhea. In two of the cases, a polypoid mass was noted in the left ear, and in one case the tumor affected the right ear. In all three cases a perforated tympanic membrane allowed the polypoid mass to be seen. In two of the reported cases,[5,6] the patients had sinonasal tract papillomas diagnosed prior to the development of their middle ear tumors. In one the sinonasal tract papilloma occurred 1.5 years prior to the middle ear papilloma, and in the other it preceded the middle ear papilloma by 9 years. The sinonasal tract tumors occurred on the same side as the middle ear tumors. At the time the patients presented with their middle ear tumors, there was no evidence of papillomas in the sinonasal tract or nasopharynx of either patient. The histologic characteristics of the middle ear tumor were apparently similar to those of the previous sinonasal papillomas. In one patient,[5] an excisional biopsy was the initial procedure, followed 6 months later by a modified radical mastoidectomy due to recurrent tumor. Further workup, including CT, demonstrated the presence of osseous destruction necessitating an extended radical mastoidectomy. A histopathologic diagnosis of inverted papilloma with areas of squamous cell carcinoma was rendered. On the basis of the presence of carcinoma, supplemental radiotherapy (55 Gy) was administered. Indications in this report were that the patient was disease-free following surgery, but no time frame was given. While a squamous carcinoma could have been present, the single photomicrograph purportedly depicting the focus of carcinoma falls short of achieving this goal. This illustration shows nests of well-differentiated squamous epithelium within the submucosa. However, this could easily represent a tangential section rather than foci of invasive squamous carcinoma. Because of poor health, the other patient was not felt to be a good surgical candidate for radical surgery.[6] The tumor was debulked to control related symptoms. According to this report, the tumor persisted, requiring periodic debulking. No time frames were reported. The third case[7] was diagnosed as an inverted papilloma and was sent to the Armed Forces Institute of Pathology for confirmation. Additional details related to this case appear in this study.

All of the cases reported in this study were in women, and all symptoms related to the left ear. The most common complaint was that of decreased hearing in the affected ear. In all of these patients there was an antecedent or concomitant history of chronic otitis media. The patients were given a course of antibiotics to treat the ear infections or myringotomy with placement of pressure-equalizing ear tubes. However, the otitis media did not resolve following treatment. Clinical evaluation revealed the presence of a middle ear polypoid tumor. The pathologic findings in all the tumors were identical to those in the schneiderian papillomas of the sinonasal tract. Of interest, most of these lesions demonstrated the histologic features of the cylindrical cell type of schneiderian papilloma, which include epithelial cells with prominent granular eosinophilic cytoplasm. However, features of inverted papilloma were also seen. None of the cases demonstrated histologic evidence of a potentially aggressive neoplasm such as epithelial dysplasia and/or invasive carcinoma. The presence of surface keratinization or dyskeratosis in a sinonasal tract schneiderian papilloma may be associated with a more aggressive lesion such as a malignant degeneration of a papilloma.[1] Neither surface keratinization nor dyskeratosis were identified in any of the cases. Mucin stains showed the presence of intracytoplasmic and extracellular mucin deposition. The immunohistochemical findings of this study showed the presence of cytokeratin and carcinoembryonic antigen reactivity, but no reactivity was seen with chromogranin.

The differential diagnosis primarily is with a middle ear adenoma. In contrast to middle ear adenomas, the schneiderian papillomas are not gland-forming tumors, nor do they demonstrate the cytomorphologic features seen in middle ear adenomas.[13] Middle ear adenomas form neoplastic glands, but also may grow in solid sheets of cells. Frequently, middle ear adenomas have a plasmacytoid cell population seen lining glandular structures or forming solid sheets. Immunohistochemical reactivity with epithelial markers such as cytokeratin would not differentiate schneiderian papillomas from middle ear adenomas, as both of these tumors are cytokeratin-reactive. This distinction remains one based on the light microscopic features. Middle ear adenomas have been reported to be reactive with neuroendocrine markers such as chromogranin.[13] There was no chromogranin reactivity seen in association with the cases of schneiderian papilloma herein reported. Other tumors occurring in the middle ear, such as jugulotympanic paraganglioma, acoustic neuroma, and meningioma, should not present diagnostic confusion with middle ear schneiderian papillomas, and should be readily distinguished on the basis of light microscopic evaluation. Recently, a middle ear and temporal bone neoplasm with invasive growth has been identified. Heffner[14] initially described these lesions as papillary adenocarcinomas of endolymphatic sac origin. More recently, Heffner has felt that a better term for these lesions is invasive papillary cystadenoma of endolymphatic sac origin (personal communication). This terminology reflects a tumor that over extended periods of time is locally and often extensively invasive but does not have metastatic capability. These tumors are characterized by the presence of an exophytic growth composed of a flattened cuboidal epithelium overlying granulation-appearing tissue. At times these tumors contain colloidlike material simulating the appearance of a thyroid neoplasm. On the basis of their histology and characteristic localization to the petrous ridge of the temporal bone, the differentiation from a schneiderian-type papilloma should not be difficult. These invasive endolymphatic sac papillary tumors have been shown to represent a component lesion of von Hippel–Lindau syndrome.[15]

Therapy for schneiderian papillomas of the middle ear is surgical. However, surgery should include at least a tympanomastoidectomy. As demonstrated by the cases in this study, conservative surgery including myringotomy and simple excision proved ineffective at controlling disease. These patients and even one other patient initially treated with a tympanomastoidectomy (case 2) had recurrent tumor over extended periods of time, and only following radical tympanomastoidectomy were the tumors completely eradicated. Therefore, a diagnosis of schneiderian papilloma of the middle ear space should prompt serious consideration of a radical tympanomastoidectomy as the initial procedure of choice. Only one patient (case 3) did not have evidence of recurrent disease. She was treated by a tympanomastoidectomy. Perhaps in her situation, the presence of congenitally malformed middle ears and eustachian tubes contributed to her resistance to recurrent disease. In two of the patients (cases 1 and 5) the primary tumor and/or recurrent disease was specifically identified at the orifice of the eustachian tube. The significance of this finding is unclear.

What is the basis for the development of these middle ear schneiderian papillomas? Stone et al,[5] in their case report, invoke the notion that their patient's middle ear papilloma resulted from transmission of the prior sinonasal tract schneiderian papilloma through the eustachian tube to the middle ear space. However, this is a remote possibility, especially in the absence of identifying nasopharyngeal papillomas colonizing the eustachian tube. Stone et al did not identify a nasopharyngeal neoplasm, nor were any nasopharyngeal tumors seen in the cases reported in this study. Further, none of our cases had a preexisting or coexisting sinonasal tract schneiderian papilloma. Kaddour and Woodhead[6] also reject the notion of transmission via the eustachian tube, suggesting a multicentric primary origin of the schneiderian papillomas. As indicated by Kaddour and Woodhead, a more plausible explanation for the occurrence of schneiderian papillomas in the middle ear space possibly relates to extensive embryological migration of the schneiderian mucosa. The schneiderian mucosa is ectodermally derived, originating from the stomodeum (primitive mouth) in the fourth week of gestation.[16] Bilateral oval thickenings of the surface ectoderm called nasal placodes develop at the end of the fourth week of gestation. With continued development of the face, the nasal placodes eventually form the nasal pits that deepen and form the primitive nasal sacs. The paranasal sinuses develop during late fetal life, forming as outgrowths of the walls of the nasal cavity.[16] The branchial or pharyngeal apparatus is composed of primitive embryonic structures that contribute greatly to the formation of the head and neck.[16] The primitive pharynx, derived from the foregut, is lined by endoderm. The endodermal lining of the first pharyngeal pouch expands into the tubotympanic recess, which gives rise to the tympanic cavity and mastoid antrum.[12] The distal portion of the tubotympanic recess expands and becomes the tympanic cavity, and the proximal portion forms the eustachian tube.[12] The primitive pharynx widens cranially where it joins the stomodeum. These structures are separated by a bilaminar membrane called the oropharyngeal (buccopharyngeal) membrane, composed of ectoderm externally and endoderm internally.[16] The proximity of these contrasting derived mucosae that will eventually line the sinonasal tract and middle ear mucosa, respectively, may result in the "ectopic" inclusion of schneiderian mucosa in anatomic sites not normally containing this mucosa, such as the middle ear. In theory, an embryological maldevelopment may result in the origin of middle ear schneiderian papillomas that retain both phenotypic expression (inverted and cylindrical cell features) and biologic behavior (recurrence) similar to those seen in the sinonasal tract. The stimulus for the development of these middle ear tumors is unknown. However, perhaps in individuals who have "ectopic" schneiderian mucosa in the middle ear, the insult may be from an ongoing otitis media resulting in squamous metaplasia of the middle ear and development of a schneiderian-type papilloma. Hyams[9] indicated that inverted papillomas enlarge by inducing squamous metaplasia of the adjacent mucosa. Chronic inflammation (sinusitis) may induce squamous metaplasia of the sinonasal tract mucosa. Similarly, the middle ear mucosa may undergo squamous metaplasia secondary to long-standing chronic otitis media, with subsequent development of a middle ear schneiderian papilloma. This theory is supported by a history of otitis media in all of the cases reported in this study.

As previously indicated, HPV has been identified in sinonasal tract schneiderian papillomas, and a possible link as an etiologic factor in the development of these lesions has been made.[10,11] Immunohistochemical evaluation for the presence of HPV in the cases reported in this study failed to detect the presence of virus. Similarly, Roberts et al[7] did not identify HPV in the report of their cases. Therefore, the possibility for HPV-induced middle ear schneiderian papillomas cannot be suggested. As HPV could not be identified in these cases, it is difficult to suggest a specific cause for these tumors. Another possible but remote theory for the development of the middle ear schneiderian papillomas could include a genetic predisposition for the development of these tumors. Two of the patients reported in the literature had separate sinonasal papillomas in addition to their middle ear schneiderian papillomas.[5,6] A genetic predisposition may explain the multicentricity of these neoplasms in these patients. However, there is no definitive proof to indict a genetic cause for these neoplasms.

This study documents the unusual occurrence of schneiderian-type papillomas of the middle ear. These tumors have a histopathologic appearance and biologic behavior similar to those of schneiderian papillomas arising in their usual location within the nasal cavity and paranasal sinuses. Similar to their sinonasal counterparts, the middle ear tumors will recur if not completely excised. Therefore, their specific morphologic recognition by the surgical pathologist is essential for the initiation of appropriate management. The latter should include complete surgical eradication in order to preempt recurrent disease. Definitive surgery may require radical tympanomastoidectomy. Malignant degeneration of these tumors has yet to be indisputably identified. However, malignant transformation, a rare complication associated with sinonasal schneiderian papillomas, is a possibility in long-standing (recurrent) middle ear schneiderian-type papillomas, and represents another indication for early aggressive surgical management.

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EXPERIMENTAL OTITIS MEDIA WITH *MORAXELLA (BRANHAMELLA) CATARRHALIS*

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Two hundred fifty gerbils and 7 chinchillas were utilized in 11 experiments to determine the effect of inoculating viable and heat-killed suspensions of *Moraxella (Branhamella) catarrhalis* into the middle ear cavity. Development of otitis media was observed by otoscopy and histopathology. Gerbils were found to be susceptible to 2×10^4 viable *M catarrhalis* cells. Depending on the number of cells inoculated, the resulting untreated inflammation was a very mild to moderately severe, self-limiting disease with no permanent sequelae except in animals inoculated with high (10^6 to 10^7) numbers of bacteria. Viable bacteria could not be isolated from the middle ears 24 hours after inflammation was induced. Heat-killed cells produced less severe acute inflammation with no permanent sequelae. We conclude 1) gerbils and chinchillas are susceptible to a self-limited inflammation caused by *M catarrhalis*, 2) no infection occurs, since viable bacteria cannot be recovered from middle ear aspirates, and 3) viable cells produce more severe inflammation than heat-killed cells.

KEY WORDS — animal model, *Branhamella catarrhalis*, chinchilla, Mongolian gerbil, *Moraxella catarrhalis*, otitis media.

INTRODUCTION

Although previously considered to be a commensal organism, *Moraxella (Branhamella) catarrhalis* now is known to produce otitis media, sinusitis, acute and chronic laryngitis, and bronchopulmonary infections.[1,2] There are numerous reports of otic disease in which *M catarrhalis* was isolated as the only organism or in mixed culture.[3-15] This species has emerged as the third most often isolated organism in cases of otitis media,[3,5,7-9,11,15] following *Streptococcus pneumoniae* and *Haemophilus influenzae*, in that order. *Moraxella catarrhalis* is an uncommon cause of meningitis, endocarditis, urethritis, conjunctivitis, keratitis, and septic arthritis[2,3,11,13,16]; however, since better identification tests are now available,[1] the true incidence of infections by this organism should become evident in future work.

Little is known of the pathogenic factors of this species,[1,11] except that it can secrete deoxyribonuclease and histamine, adheres to human cells, and possesses membrane-associated proteins that bind human lactoferrin and human transferrin. Many strains of *M catarrhalis* are known to produce a β-lactamase, thereby making treatments of mixed infections with lactam ring antibiotics ineffective in the presence of the organism.[3,7-11,13,14] This has established the concept of passive or indirect pathogenicity by the organism in mixed infections.[16-18]

At our university, studies have shown the Mongolian gerbil, *Meriones unguiculatus*, to be a useful model for experimental otitis media.[18-23] Our studies[21,22] have shown the gerbil to be susceptible to otitis media caused by nontypeable *H influenzae*[24] and *S pneumoniae* type 3. These organisms caused striking and permanent pathologic sequelae in the gerbil middle ear, similar to those seen in the mastoid air cells of humans with untreated disease.[25-29] Other strains of these species produced a milder form of the disease with less severe sequelae.[20,30] Species of anaerobic bacteria produced moderate to severe otic disease in gerbils, depending on the species and on the mixtures of species inoculated.[31]

Recently, Chung et al[32] reported the results of inoculating high doses of *M catarrhalis* into chinchilla middle ear cavities. They found that inflammation resulted and that viable *M catarrhalis* could be isolated from the inoculated ears for up to 5 days, suggesting that current animal models of otitis media may not be appropriate for study of *M catarrhalis*, since the organisms are rapidly eliminated.

The purposes of the present study were to determine the lowest dose of *M catarrhalis* necessary to cause otitis media in the gerbil, to compare otitis media in the gerbil caused by living cells of *M catarrhalis* with otitis media caused by heat-killed cells, to undertake a longitudinal study of the pathology of untreated *M catarrhalis* otitis media in the gerbil, and to compare the disease in gerbils with that in chinchillas.

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TABLE 1. SCHEDULE OF EXPERIMENTS

Experiment	*Date*	*Purpose*	*Materials or Level of Organisms Injected*	*No. of Animals*	*Data Collected*
9	April 1987	Compared injected carrier vehicles	Air	2	2, 5, or 7 days
			DW	2	
			NPSS	4	
			SS	5	
			TH	5	
10	May 1987	Preliminary determination of effect of viable cells in NPSS	NPSS	3	2, 5, 7, 10, or 14 days
			7.2×10^5	5	
			7.2×10^6	5	
			7.2×10^7	5	
11	June 1987	Final definitive experiment	NPSS	3	3 and 7 days; weekly thereafter up to 5 weeks
			3.6×10^4 HK	5	
			2.3×10^4 Viable	10	
			3.6×10^5 HK	5	
			2.3×10^5 Viable	10	
			3.6×10^6 HK	5	
			2.3×10^6 Viable	10	
			3.6×10^7 HK	5	
			2.3×10^7 Viable	10	
		Chinchilla experiment	1.4×10^3	7	4, 7, 18, and 25 days

Results of experiments 1 through 8 are not reported. See Materials and Methods.

DW — deionized, sterilized water, NPSS — nonpyrogenic sterile saline (commercial preparation), SS — sterile saline, TH — Todd-Hewitt broth (bacteriologic growth medium), HK — heat-killed cells of *Moraxella catarrhalis*, viable — living cells of *M catarrhalis*.

MATERIALS AND METHODS

Table 1 outlines the experiments reported here with *M catarrhalis*. Because our preliminary experiments (1 through 8) were confounded by variability, later determined to be due to contamination of our deionized water with endotoxin, as evidenced by subepithelial hemorrhage in ears[33] injected with our deionized water, we report here only the results of experiments 9, 10, and 11.

Animals. Healthy young adult (less than 6 months of age) Mongolian gerbils, *M unguiculatus*, and chinchillas of undetermined age were housed, maintained, anesthetized, and examined as in our previous studies.[20-22,31] Animals were fed NIH 07 rodent diet with water ad libitum.

Bacterial Cultures. The strain of *M catarrhalis* used in this study was originally purchased for our department culture collection from American Type Culture Collection (ATCC); however, the ATCC number of this strain was lost. It was one of the six ATCC strains unable to produce β-lactamase. Following our study reported here, this strain has been reaccessioned by ATCC as strain number 51584.

The organism was inoculated into mice to enhance pathogenicity and reisolated from the mouse liver. The bacterium was then recharacterized to prove its identity. To obtain sufficient numbers of bacteria, *M catarrhalis* was grown on chocolate agar supplemented with hemin and nicotinamide adenine dinucleotide. The resulting growth was harvested by washing plates with commercially prepared pyrogen-free saline for intravenous injection. (However, the preliminary experiments 1 through 8 utilized laboratory-prepared sterile saline made of deionized water.) The resulting suspension of cells was diluted to a turbidity equivalent of a No. 4 McFarland standard, providing approximately 1.2×10^9 cells per milliliter. Tenfold serial dilutions of this suspension were made in commercially prepared pyrogen-free saline for intravenous injection. Plate counts were made from dilution tubes to ascertain viable cell counts.

Inoculations. Suspensions prepared as above were inoculated in aliquots of 0.03 mL percutaneously into middle ear bullae by the method used in our previous studies.[20-22,31] Right ear bullae were inoculated, the left serving as controls. Animals were held untreated and examined according to the experimental designs listed below.

Examination of Animals. Otoscopic examinations were made with an operating microscope as described earlier.[20-22,30,31,34] To score the otoscopic observations we utilized our previously published scoring system of five categories ranging from very mild inflammation (score 1) to a highly inflamed, bulging tympanic membrane (TM) with opaque exudate (score 5).[21]

Specimens and Histologic Preparations. These were done as in our previous studies,[19-22,30,31] in which animals were perfused with saline and then 10% formalin in phosphate buffer. Heads were re-

TABLE 2. INCIDENCE OF DISEASE SEVERITY EVALUATED HISTOLOGICALLY AT INTERVALS AFTER INOCULATION (ALL DOSES)

Spectrum of Disease Severity	*Day* 2	3	5	7	10	14	21	28	35
Acute	3								
Subacute		6	3	6	2	2			1
Chronic		2		4	6	1	2	3	2
Healed with sequelae						4	3	1	3
Healed (minimal sequelae)				1	3	5			
Animals positive/animals examined	3/3	8/8	3/3	10/11	8/11	7/12	5/5	4/4	6/6
Controls positive/controls examined	0/1	0/1	0/1	0/2					

Table includes animals in experiments 10 and 11 combined (total inoculated with *Moraxella catarrhalis* was 63).

Acute — predominance of polymorphonuclear cells and absence of granulation tissue and bone changes; chronic — predominance of mononuclear cells, presence of fibrosis and/or bone changes; subacute — presence of signs of both acute and chronic disease; healed — no evidence of active disease; positive — positive histologic signs of disease.

moved, decalcified, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The type and severity of infection, as well as sequelae, were evaluated by grading or scoring the volume of inflammation, the cellular composition of the inflammation, the presence or absence of mucoperiosteal elevation, the amount of granulation tissue, and the degree of bone change. These results were summarized for each animal by recording as mild, moderate, or severe and as acute, chronic, or, where features of both acute and chronic disease were seen, acute and chronic.

Comparison of Effects of Inoculated Heat-Killed Cells With Effects of Inoculated Viable Cells. As shown in Table 1, experiments 10 and 11, groups of gerbils were each inoculated, respectively, with different concentrations of heat-killed or viable *M catarrhalis* cells. Animals were examined at days postinoculation as indicated in Table 1. These experiments were run in parallel to the experiments investigating the infective dose levels. The heat-killed cells were heated to 80°C for 10 minutes to kill the bacteria. All animals were observed otoscopically, and 1 animal was chosen at random for sacrifice for histologic study at each time period.

Controls. Three groups of animals were inoculated with 0.03 mL commercially prepared pyrogen-free intravenous saline as controls.

Interpretation of Disease. Interpretation of disease was made on the basis of histopathologic findings seen in tissue sections, rather than on judgment of otoscopic observations. Acute disease was defined as the predominance of polymorphonuclear cells (PMNs) and the absence of granulation tissue and bone changes. Chronic disease was defined as the predominance of mononuclear cells, the presence of fibrosis, or the presence of bone changes. In cases in which aspects of both acute and chronic disease were seen, the term subacute was used (Table 2). The inflammation, whether acute, chronic, or both acute and chronic in appearance, was graded as mild, moderate, or severe. In addition, the presence of mucoperiosteal elevation was noted.

Chinchilla Inoculations. Seven chinchillas were inoculated with 1.4×10^3 viable *M catarrhalis* cells in the same manner as described by Giebink et al.[35] Otoscopic and histologic examinations were made as above.

Serény Test. To determine the invasiveness of our strain of *M catarrhalis*, the Serény test[36] for invasiveness was performed by instilling 0.3 mL of a suspension of 1.2×10^9 *M catarrhalis* into the conjunctival sac of the eyes of 2 guinea pigs, 10 gerbils, and 2 chinchillas. Animals were observed for conjunctivitis and also for invasion of the cornea as evidenced by opacity of the cornea in the living animal.

RESULTS

Normal Middle Ear Tissues of Gerbil. Descriptions and photomicrographs of normal gerbil middle ear tissues and structures have been published elsewhere.[19,35,37]

Control Animals. Inoculating sterile, nonpyrogenic intravenous saline had only a slight effect on the gerbil middle ear. The TMs of 2 animals showed very slight inflammation, while the others were normal. Histologic examination showed very slight thickening of the periosteum of these control animals at the fifth day postinoculation. All other control animals appeared normal.

Serény Test. The Serény test, as a test of invasiveness of *M catarrhalis* in gerbils, chinchillas, and guinea pigs, was negative for conjunctivitis and negative for invasion of the cornea, indicating that *M catarrhalis* is not an invasive organism in these animal species.

Reisolation of Inoculated Organisms From Middle Ear Cavities. With the exception of 1 animal, no viable cells of *M catarrhalis* were recovered from the specimens of aspirated middle ear fluids, the aspirated saline rinses, or the tissue specimens from the gerbils tested after 24 hours following inoculation.

Infective Dose. The lowest dose found to cause disease in the gerbils was 2 × 10^4 cells per ear, and at this dose, viable cells produced greater histopathology than dead cells. Since we were unable to isolate viable bacteria from inoculated ears, the term inflammatory dose is more appropriate than infective dose to describe these studies.

Longitudinal Study by Otoscopic Examination. In gerbils inoculated with *M catarrhalis*, otitis media was present from the first otoscopic observation at day 2 and persisted at least through day 10. Similar results were obtained by using dead organisms at the same inoculum levels. Otoscopic study revealed a very mildly inflamed pars flaccida with slight opacity or a rough surface appearance (score 1 to 2). In many animals a transparent to translucent exudate was seen behind the pars flaccida, around the malleus. In some animals the fluid had a yellow color and the malleus also appeared yellow (score 2). Animals receiving doses of inoculum higher than 2 × 10^4 viable cells showed additional opacity to the pars tensa of the TM, with a whitish or grayish coloration and increased vascularity (score 3 to 4). By the end of the first week, all animals receiving less than 2 × 10^6 cells exhibited a TM showing very mild inflammation (score 1), and this persisted as a roughened TM surface after the increased vascularity had subsided. Most animals showed the rough surface appearance of the TM throughout the study, while the TMs of others returned to normal. This return to normal occurred beween day 10 and week 2 postinoculation and was random among the groups at inoculum levels below 2 × 10^6 cells. Animals receiving higher numbers of living cells (2 × 10^6 and 2 × 10^7) exhibited changes in the TM, scored as 2 to 4 (moderate to severe inflammation, opaque TM with much exudate), that persisted beyond week 4 of the study. Animals receiving 2 × 10^6 and 2 × 10^7 dead cells were studied only for 2 weeks. These animals had only very slight changes in the TM after 10 days.

Longitudinal Study by Histopathology. Animals inoculated with 2 × 10^4 (living or dead) or fewer organisms showed at 3 days a very mild otitis with both acute and chronic characteristics at 3 days. Mucoperiosteal elevation and a small amount of exudate containing both PMNs and mononuclear cells were present. At 7 days granulation tissue and slight bone change were noted in 1 animal; another showed almost no change. By 10 days all animals receiving this level of inoculum appeared normal, exhibiting a very small amount of exudate containing mainly mononuclear cells. After 10 days all animals appeared normal.

Animals inoculated with 2 × 10^5 living organisms at day 3 had moderate acute and chronic disease with mucoperiosteal elevation and edema, some granulation tissue, and some areas of mild bone change. The volume of exudate was greater than in animals inoculated with dead organisms at this same level or with living organisms at lower levels. At day 7 animals showed less exudate and some granulation tissue. There was edema in the mucoperiosteum of animals receiving 2 × 10^5 dead organisms. At 10 days animals were almost normal, and from day 14 through day 35 all animals were normal.

All animals receiving 2 × 10^6 or 2 × 10^7 (living or dead) cells per ear showed moderate acute and chronic inflammation at day 3. Moderate exudates filling half or more of the middle ear cavity contained more monocytes than PMNs. Periosteal elevation with mild to moderate fibrosis was seen. Bone changes of resorption and deposition were seen in animals receiving living organisms. At day 7 the disease continued as a mild acute and chronic disease. The animals receiving 2 × 10^7 cells per ear had severe chronic disease at day 10, including many mononuclear cells, granulation tissue, and moderate bone changes. These changes persisted throughout the remainder of the study (to day 35 postinoculation) in animals receiving 2 × 10^6 or 2 × 10^7 living organisms per ear, although the degree of changes indicated moderate chronic disease from day 14 through day 35.

All except 1 of the animals inoculated with living or dead *M catarrhalis* and examined prior to 7 days postinoculation showed evidence of subepithelial hemorrhage or red blood cells in the exudate.

The changes described are illustrated as follows. Table 2 shows the incidence of disease severity with all doses of *M catarrhalis* at intervals following inoculation. Figure 1 is typical of animals inoculated with 2 × 10^5 or more living *M catarrhalis* at 3 days, showing considerable exudate with a preponderance of PMNs and a thickened, vascularized mucoperiosteum with some granulation tissue, but no bone changes. At 7 days postinoculation all animals receiving 2 × 10^5 or higher doses per ear had a thickened mucoperiosteum with little or no bone change, and

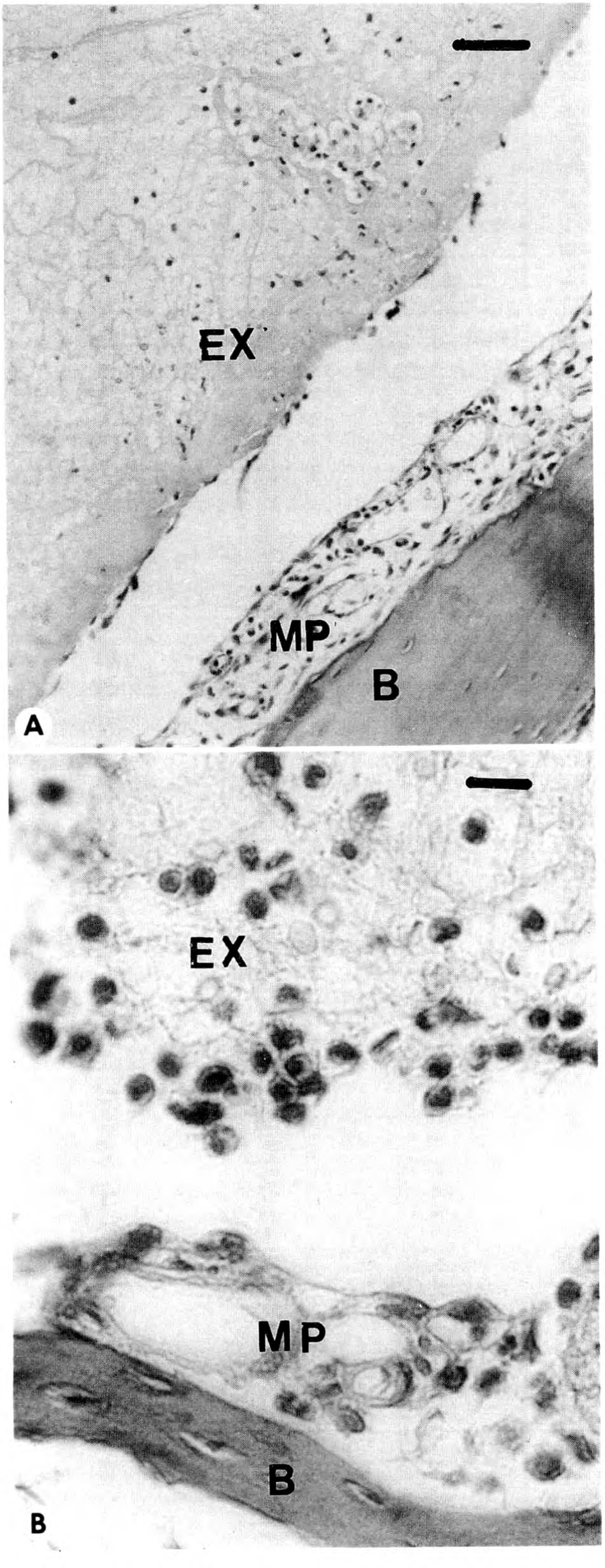

Fig 1. Histopathology of gerbil middle ear 3 days after infection with *Moraxella (Branhamella) catarrhalis*, showing normal bone (B), thickened mucoperiosteum (MP), and purulent exudate (EX) containing polymorphonuclear leukocytes. A) Original ×125 (bar — 50 μm). B) Original ×500 (bar — 10 μm).

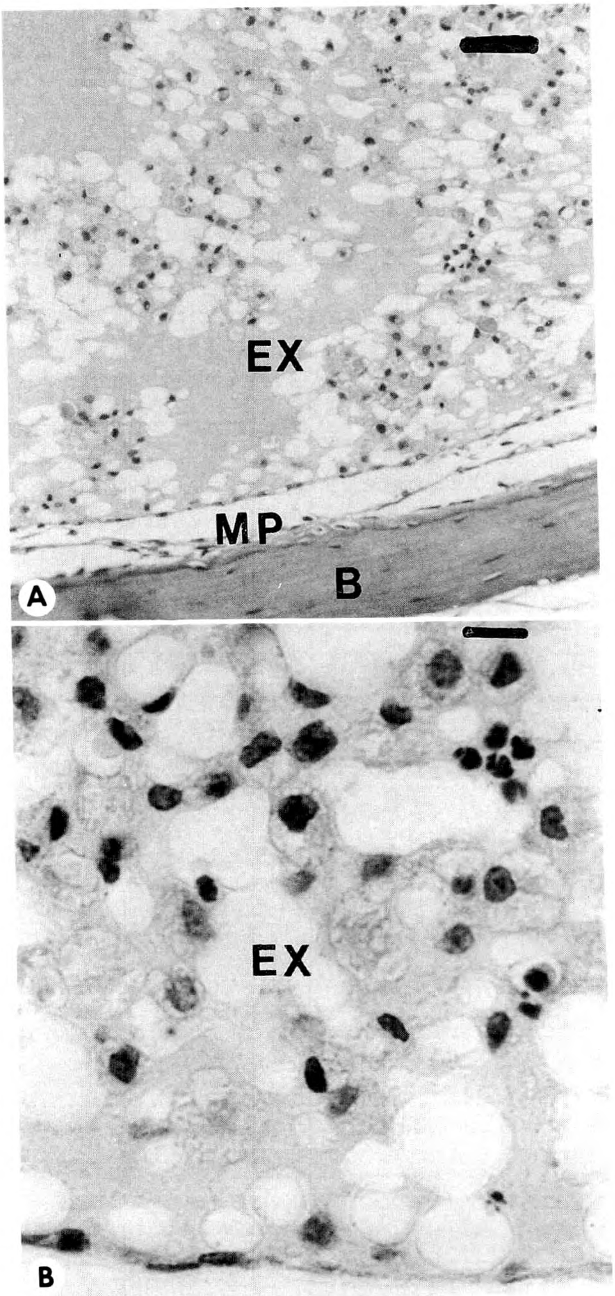

Fig 2. Histopathology 7 days after infection with *M catarrhalis*, showing bone (B), edematous mucoperiosteum (MP), and exudate (EX) containing polymorphonuclear leukocytes and macrophages. Elevation of MP was due to edema; however, MP also may have been slightly displaced from bone as processing artifact. A) Original ×125 (bar — 50 μm). B) Original ×500 (bar — 10 μm).

exudates containing both PMNs and mononuclear cells. This is seen in Fig 2. Animals receiving 2×10^6 or 2×10^7 organisms per ear showed changes in bone illustrated in Fig 3 after 7 days postinoculation. Granulation tissue was often seen in these animals after 2 weeks as illustrated in Fig 4. Animals receiving 2×10^5 or less organisms appeared to recover with

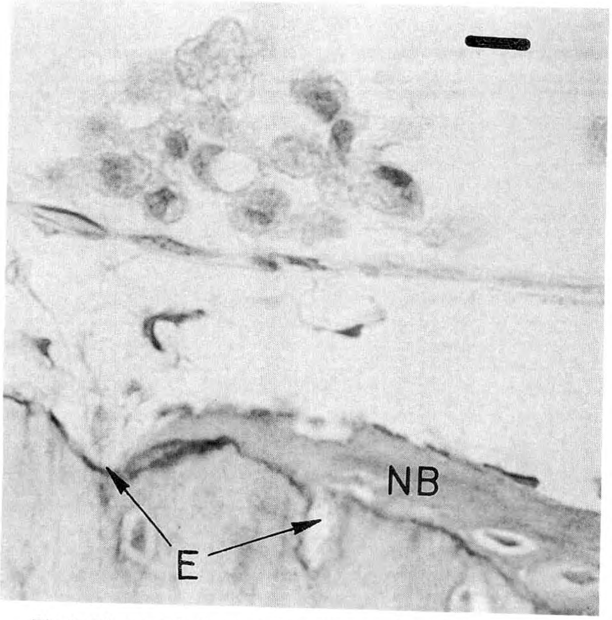

Fig 3. Changes in bone at 2 weeks postinoculation with *M catarrhalis*, showing bone erosion (E) and new bone deposition (NB). Original ×500 (bar — 10 μm).

few sequelae. Figure 5 illustrates a mildly thickened mucoperiosteum in 1 of these gerbils 56 days postinoculation as the only discernible change.

DISCUSSION

In the present study, *M catarrhalis* has been shown to produce in gerbils otic disease of severity much less than that of the more virulent pathogens, such as *H influenzae* (nontypeable) and *S pneumoniae* type 3.[20-22,30] *Moraxella catarrhalis* at 2×10^4 cells can produce experimental otitis media; however, it is unlikely that this organism reaches these numbers in the normal ear. Undoubtedly, *M catarrhalis* exerts its effects as a secondary invader, following predisposition of the middle ear mucoperiosteum by other factors that are able to cause otitis media, such as viral or other bacterial infections.

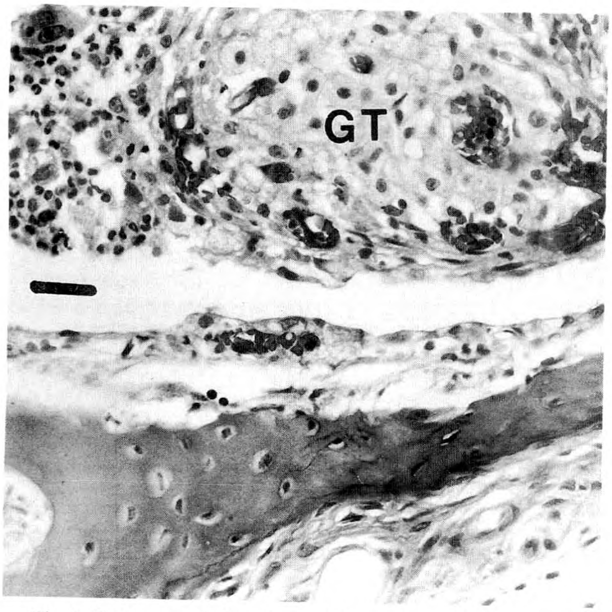

Fig 4. Early granulation tissue (GT) with capillary proliferation and leukocytes at 7 days after inoculation with *M catarrhalis*. Original ×400 (bar — 12.5 μm).

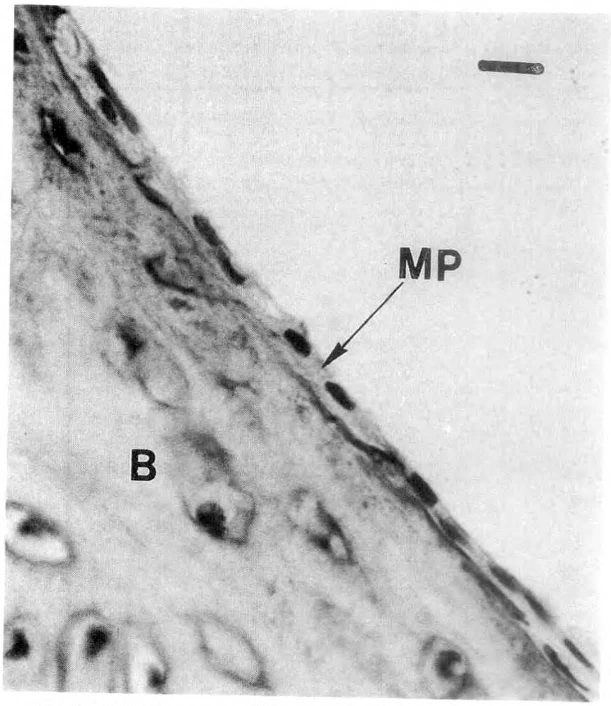

Fig 5. Thickened but otherwise normal mucoperiosteum (MP) from gerbil that recovered from middle ear inoculation of *M catarrhalis*. Eight weeks postinoculation. Original ×500 (bar — 10 μm). B — bone.

Earlier experimental otic infections using *M catarrhalis* in Mongolian gerbils[38] suggested that as few as 390 living cells of *M catarrhalis* could initiate otitis media in gerbils. It was subsequently found that the carrier saline in which the inoculum was suspended contained small amounts of pyrogens that caused the inflammation. Otherwise, the findings were similar to those of the present study, except that the present study more accurately defines the concentration of organisms necessary to achieve inflammation to be 2×10^4 organisms rather than 2×10^2.

Experimental otic infections using *M catarrhalis* were carried out in chinchillas by Chung et al[32] and by Doyle.[39] Chinchillas were inoculated with very high doses (0.3 mL of a 1×10^9 suspension) of the organisms.[32] The results of these studies were similar to the results we report here, except that *M catarrhalis* survives longer in chinchillas.

In this study we have shown that the middle ear of the gerbil, when inoculated with large numbers of *M catarrhalis*, exhibits inflammation and pathologic changes (inflammatory cells, granulation tissue, and ossification) similar to those seen in the middle ear of humans.[25-29] As shown by this study, *M catarrhalis* is able to cause inflammation in the gerbil with an inoculum of 2 × 10^4 or more cells. The histologic changes have been described and are depicted in Fig 2. They involve an acute phase that lasts 3 to 4 days and is defined histologically by the presence of a neutrophilic exudate. A chronic phase supervenes and manifests itself by the presence of macrophages, granulation tissue, ossification, and bone remodeling. Higher doses of organisms shortened the acute phase and lengthened the chronic phase. Heat-killed organisms caused otitis media, although the disease was somewhat milder than that caused by living organisms injected in the same concentration.

We have defined acute otitis media and chronic otitis media in this study on a histologic basis, using accepted criteria based on inflammatory cell type, fibrosis, and ossification. Definitions of acute and chronic otitis media in the otologic literature are based on otoscopic observations and other clinical criteria. Our results suggest that chronic changes, as defined histologically, may be occurring much earlier than presumed by the clinical definitions of acute and chronic otitis media. Our results also suggest that these histologic changes, ie, fibrosis and bone formation, are not always reversed over the time course of 6 weeks.

In a previous study using Koch's postulates, we proved that the gerbil is susceptible to acute, experimental otitis media due to *S pneumoniae* and *H influenzae*.[20] With *M catarrhalis* we were unable to recover living bacteria from the experimental infections, and consequently we were unable to satisfy Koch's postulates. However, all gerbils inoculated with this organism in the right ear developed otitis media in the right ear, while the uninoculated left ear, serving as a control, remained normal. This inflammation undoubtedly was due to endotoxin and to cell wall components that cause similar inflammation in gerbils and chinchillas (manuscript in preparation).

The finding of a slightly thickened (to two or three layers of cells) mucoperiosteum in 2 of 10 gerbils inoculated with commercially prepared, pyrogen-free saline for human injection was unusual. In previous studies this observation was rarely made,[20-22,30,31] and animals inoculated with saline showed entirely normal ears. However, the slightly thickened mucoperiosteum reported in these 2 animals was trivial in comparison to the results of inoculating *M catarrhalis*.

It is important to exclude confounding effects of endotoxin that may be induced by saline solutions made in the laboratory. For example, we have found that sterile saline made from high-quality deionized water may cause inflammation similar to that caused by 1 × 10^5 organisms (unpublished data). This effect has been also reported in other papers.[40] The use of commercially prepared pyrogen-free saline for human injection greatly reduces the effect of control injections, as well as underlying inflammation, when pathogenic organisms are injected; therefore, it establishes a better baseline for experimental infections and a clearer picture of the inflammation elicited by the organisms injected.

The finding of subepithelial hemorrhage or red blood cells in the exudates of the acute phase of the experimental disease with *M catarrhalis* is to be expected. *Moraxella catarrhalis* is a gram-negative organism that produces an outer membrane. This outer membrane consists of short-chain oligosaccharide attached to a lipid A moiety. The lipid A moiety of *M catarrhalis*, the toxic moiety of outer membrane or endotoxin, contains a backbone of β-1,6-D-glucosamine.[41] We have shown that similar components of peptidoglycan can cause bleeding and subepithelial hemorrhage in the middle ears of gerbils (manuscript in preparation).

We conclude 1) gerbils and chinchillas are susceptible to a mild, self-limited inflammation caused by *M catarrhalis*, 2) no infection occurs, since viable bacteria cannot be recovered from middle ear aspirates, 3) viable cells produce more severe inflammation than heat-killed cells, and 4) the gerbil and the chinchilla are not useful models of infection caused by *M catarrhalis*, although these animals exhibit inflammation and sequelae due to inoculation of *M catarrhalis*.

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IMAGING CASE STUDY OF THE MONTH

HUMAN IMMUNODEFICIENCY VIRUS INFECTION PRESENTING WITH LYMPHOEPITHELIAL CYSTS IN A SIX-YEAR-OLD CHILD

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Lymphoepithelial cysts associated with human immunodeficiency virus (HIV) infection have been described in adults.[1-3] Approximately 6% of adult HIV-infected patients have parotid swelling.[4] In children, however, there are fewer reports of parotid swelling and cysts. It has been reported that approximately 10% of children with HIV disease have parotid enlargement.[5] We present a case report of a 6-year-old child whose initial presentation of HIV was cystic enlargement of the parotid glands. The purpose of this article is to raise the awareness of practitioners regarding the association of parotid enlargement and HIV disease in children.

CASE REPORT

A 6-year-old girl had a 1-year history of painless parotid swelling bilaterally. There was no history of previous infection or trauma and the patient had been in good health. The patient had been to several practitioners and carried the diagnosis of Epstein-Barr virus infection. She was referred to the otolaryngology clinic.

The mother stated the glands had been slowly enlarging. There were no complaints of pain, dry mouth, or difficulty with chewing or swallowing.

Physical examination revealed 4 × 5-cm masses in the region of both parotid glands with no overlying erythema (Fig 1). On palpation the masses were soft and nontender, with a fluid-filled consistency. Flow from Stensen's ducts was clear. There was no trismus and facial nerve function was normal. Bilateral cervical adenopathy was seen.

A computed tomography scan was obtained that showed multiple cysts contained within each parotid gland, within both the superficial and deep lobes (Fig 2).

Fine needle aspiration was performed. Approximately 15 mL of clear yellow fluid was aspirated from each gland. Cultures of this fluid were negative. Cytology showed a proteinaceous background with a few atypical lymphoid cells and a few histiocytes present. The following laboratory values were obtained from the aspirated fluid: amylase 1,365 IU/L, protein 7,460 mg/dL, lactate dehydrogenase 994 U/L, and glucose 31 mg/dL.

The patient subsequently underwent an HIV test and was positive. The mother denied any risk factors or present illness. No other family members were known to be HIV-positive.

DISCUSSION

Etiology. The origin of lymphoepithelial cysts is controversial. In the embryo at 5 weeks' gestation, lymph nodes are trapped within the capsule of the

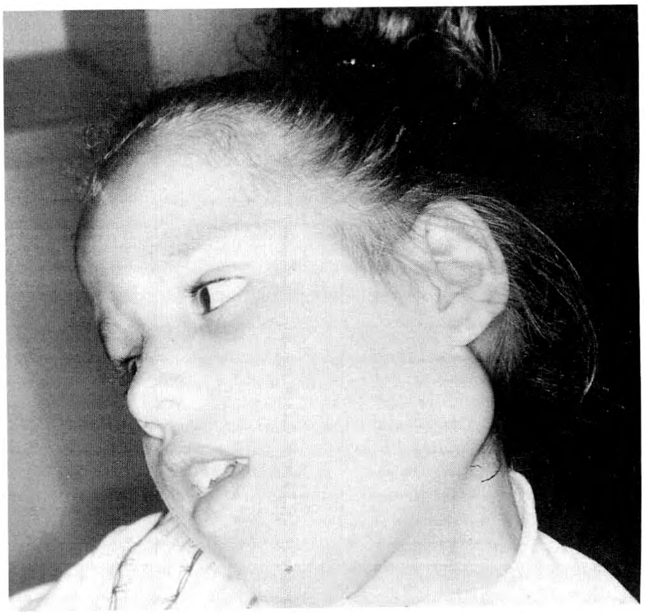

Fig 1. Six-year-old girl with bilateral parotid gland enlargement demonstrating mass of left parotid.

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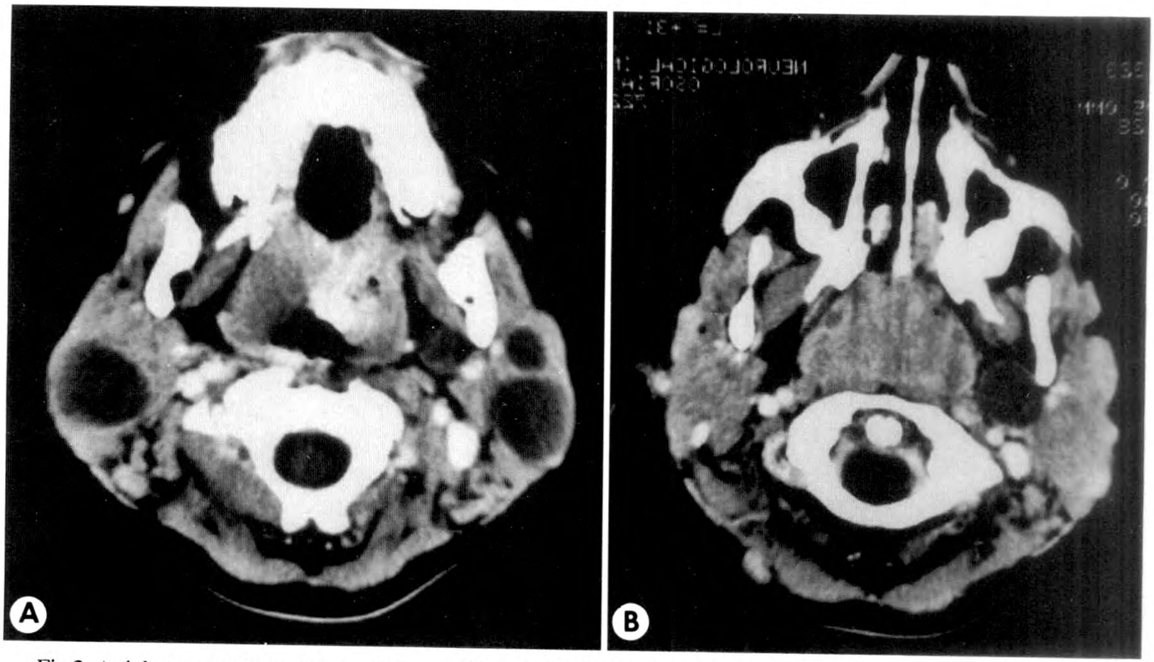

Fig 2. Axial computed tomograms of 6-year-old girl with bilateral parotid enlargement. A) Demonstrating bilateral superficial parotid cysts. B) Demonstrating cystic enlargement of deep parotid lobe.

parotid gland. It is known that there are salivary inclusions within these lymph nodes.

In HIV infection, lymphoid hyperplasia occurs in all lymphatic tissue; it is suggested that lymphoid hyperplasia in intraparotid lymph nodes obstructs salivary ducts and leads to cystic enlargement.[6] If this were the case, one might expect pain and intermittent swelling to be associated with these cysts.

Another, more accepted theory[7] is that salivary elements trapped within hyperplastic intraparotid lymph nodes become hyperplastic themselves, and epithelial proliferation occurs with cyst formation. This theory is supported by pathologic evidence that most lymphoepithelial cysts occur within intraparotid lymph nodes.

Diagnosis. Bilateral, painless, slowly enlarging masses in the region of the parotid in children should alert the practitioner to the possibility of HIV-related parotid cysts. The past medical history may reveal recurrent infections (otitis media, tonsillitis), thrush, or a previously positive HIV test. Risk factors and the HIV status of the parents should be documented.

Palpation generally reveals soft, nontender, fluctuant masses associated with diffuse cervical adenopathy. Imaging studies may be helpful in diagnosis and as a means of evaluating the number and size of cysts.[5] Ultrasonography may be preferred over computed tomography because it is less invasive and less expensive and generally does not require sedation in children. Fine needle aspiration is a simple method for tissue diagnosis of lymphoepithelial cysts. The typical findings are a combination of small and large lymphocytes along with macrophages and histiocytes dispersed on a proteinaceous background. Open biopsy should be reserved for those cases in which the diagnosis remains obscure. Testing for HIV should be done in all children with parotid cysts.

Differential Diagnosis. Lymphoma should be considered in the differential of bilateral masses in children, although these masses are generally solid. Warthin's tumors may have a cystic component, but are infrequently bilateral.

Other cysts may occur in the parotid, including first branchial cleft cysts; these are infrequently bilateral, and often have tracts associated with the external auditory canal.

Treatment. Options for treatment of lymphoepithelial cysts are repeated aspiration, a course of zidovudine (azidothymidine; AZT), radiotherapy, and surgery. Most experience has been in adults.

Repeated aspiration is a simple office procedure that is quick and relatively well tolerated in the pediatric population. The disadvantages are that the cysts always recur within weeks to months, requiring frequent aspirations; in addition, the multicystic nature of the disease makes it difficult to completely evacuate all cysts.

A course of AZT may be beneficial in reducing or eliminating the cysts. This therapy does not succeed in all patients, and one is faced with the potential untoward effects of AZT.[2]

Low-dose radiotherapy is an effective method of reducing the size of the lymphoepithelial cysts.[2] Side effects from 800 to 2,000 cGy are minimal. However, repeated visits are required and recurrence is reported.

Superficial parotidectomy will effectively remove the cysts in the superficial lobe of the parotid gland. Cysts that occur in the deep lobe will remain and continue to enlarge. In addition, facial nerve function is placed at risk.

Interstitial laser ablation of the cysts has met with excellent success in eliminating the cysts and recurrence.[8] It is less invasive than superficial parotidectomy and more successful than repeated aspiration or radiotherapy. The downside is the investment required in purchasing the laser and the necessary equipment.

Intraluminal injection of tetracycline as a sclerosing agent has proven to be effective in limited trials.[9]

CONCLUSION

Of children with acquired immunodeficiency syndrome, 82% receive their diagnoses by 3 years of age.[10] Parotid enlargement may occur in children with HIV anywhere from 4 months to 4½ years after diagnosis. Parotid lymphoepithelial cysts have been reported as a presenting diagnosis in only one previous instance, in a 6-month-old child.[5]

We report a 6-year-old child in whom lymphoepithelial cysts were the presentation of HIV disease. Parotid enlargement in a child should alert the practitioner to the possibility of HIV-related disease, and appropriate testing should be done.

Treatment options should be individualized to the patient with consideration of the level of invasiveness, inconvenience to the patient, and rate of recurrence complications.

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CLINICOPATHOLOGICAL CONSULTATION

"EARLY" CANCER OF THE LARYNX: THE CONCEPT AS DEFINED BY CLINICIANS, PATHOLOGISTS, AND BIOLOGISTS

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Since a great deal of confusion surrounds the different uses made by clinicians, pathologists, and biologists of the term, this paper clarifies the concept of "early" cancer of the larynx. Clinically, this is usually a glottic neoplasm in which full cordal mobility is still present; early supraglottic cancer is infrequent, and usually a chance finding. Whatever the site, early laryngeal cancer is a minimally invasive neoplastic lesion that does not invade the muscle or cartilage, but is still capable of metastasis. Being confined to the lamina propria, it is more than a carcinoma in situ but less than a deeply infiltrating carcinoma. Superficial extending carcinoma is therefore an early cancer. Biologically, early cancer belongs to stage II.

The term "early" cancer was originally used in the Japanese literature to indicate a malignant lesion limited within the mucosa or submucosa of the stomach ("early" gastric cancer or "superficial" cancer). Since then, this term has been used for other organs, such as esophagus, uterine cervix, etc.

In the larynx, early cancers are usually located in the glottis. In the supraglottis and hypopharynx they are detected only infrequently and usually by accident. By way of perspective, in the United States, some 11,600 new cases of laryngeal carcinoma will be diagnosed in 1995, an incidence that roughly parallels that of carcinomas of the mouth, carcinomas of the esophagus, or multiple myeloma in this patient population.[1]

But before addressing the concept of "early" cancer of the larynx, the term should be defined, since there is an enormous amount of confusion in the literature surrounding the usage of the term "early" in relation to laryngeal cancer. As will be apparent from the discussion below, clinicians and pathologists have in some instances employed the diagnosis of "early" laryngeal carcinoma in quite different contexts — an overlap in terminology to which this article attempts to bring some degree of uniformity.

DeSanto and Olsen[2] consider that early glottic cancer includes Tis and T1 lesions — that is, tumors corresponding to the TNM stages 0 and I, respectively. These authors recognize, however, that not all T1 lesions will behave in a similar fashion to one another — extremely large T1 lesions, for example, may as a group behave more aggressively (perhaps following a course more reminiscent of the T3 lesions) than a group of very small, minimally invasive T1 lesions. C. W. Vaughan (personal communication, 1993) also notes that up to 20% of clinical T1 cancers with normal cord mobility invade the vocal ligament or the conus elasticus and beyond — "sometimes far beyond." It appears, therefore, as though the simple clinical staging of a glottic carcinoma may not always accurately predict the clinical behavior of a given lesion. These cancers have the ability to penetrate the lymphatic system and as a consequence are at risk for the development of cervical nodal metastases.[3] By way of another example, most otolaryngologists have encountered patients in whom a carcinoma located in the vicinity of the anterior commissure was evaluated clinically and staged as a T1 lesion, but in whom subsequent pathologic evaluation of a laryngectomy specimen revealed microscopic invasion of adjacent cartilage or extralaryngeal soft tissues — a finding that forced reclassification of the carcinoma as a T4 lesion.

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COMPARISON OF RECOMMENDED CLINICAL AND PATHOLOGIC USAGE OF TERM "EARLY INVASIVE CARCINOMA OF LARYNX"

	Clinical Usage	*Pathologic Usage*
Includes	T1 (stage I) lesions	Invasive squamous carcinoma that is confined to lamina propria
Excludes	Tis, T2, T3, T4 lesions	Squamous dysplasia or carcinoma in situ; carcinoma invading adjacent muscle, cartilage, or extralaryngeal soft tissues (deeply infiltrating carcinoma)
Subtypes		Microinvasive; superficial extending; multifocal; type not further subclassified

Thomas et al believe that "early glottic carcinomas present along a continuum from the microscopic focus of in situ disease to the more extensive carcinomas involving both cords without cord fixation."[4(p264)] Mendenhall et al[5] consider as early glottic cancer Tis, T1, and also T2 lesions. It is, therefore, apparent that various lesions (Tis, T1, T2, T3, and T4) have been included under the generic term of "early" tumors.

The *clinical definition* of early cancer consequently varies in significance among different clinicians. But all laryngologists agree that full cordal mobility should be present in early glottic cancer. Fixation is usually the result of thyroarytenoid muscle invasion. Partial fixation or partial mobility is synonymous with muscle invasion. The risk of neck metastasis is usually absent in true early glottic cancer. Early supraglottic cancer may be asymptomatic and may be more advanced than it clinically appears. Of course, its behavior is different from that of early glottic cancer.

The *pathologic definition* of early invasive cancer describes a minimally invasive neoplastic lesion that does not extend into the adjacent muscle or cartilaginous structures, but that is still capable of metastasis. When infiltration has reached the muscle or cartilage, the lesion is no longer an early invasive cancer, which is necessarily confined to the lamina propria.[6] Muscle infiltration coincides with a "classic" infiltrating neoplasm. Early invasive cancer is always a superficial lesion in pathologic terms; it may be extensive, albeit without muscle or cartilaginous invasion. Such extensive early invasive carcinomas are also called "carpet" carcinomas.

The early invasive carcinomas should be distinguished, both clinically and pathologically, from the earliest step along the cancer continuum that is recognizable by the light microscopist — namely, "carcinoma in situ."[7-9] The diagnosis of carcinoma in situ should be restricted to lesions of the epithelium that have not transgressed the basement membrane and therefore do not involve the lamina propria and have no metastatic potential.

As is well known to all pathologists, the usually random orientation of tiny biopsy specimens of mucosal tissue for histologic sectioning sometimes produces significant problems in interpretation — specifically, in the area of distinguishing in situ from

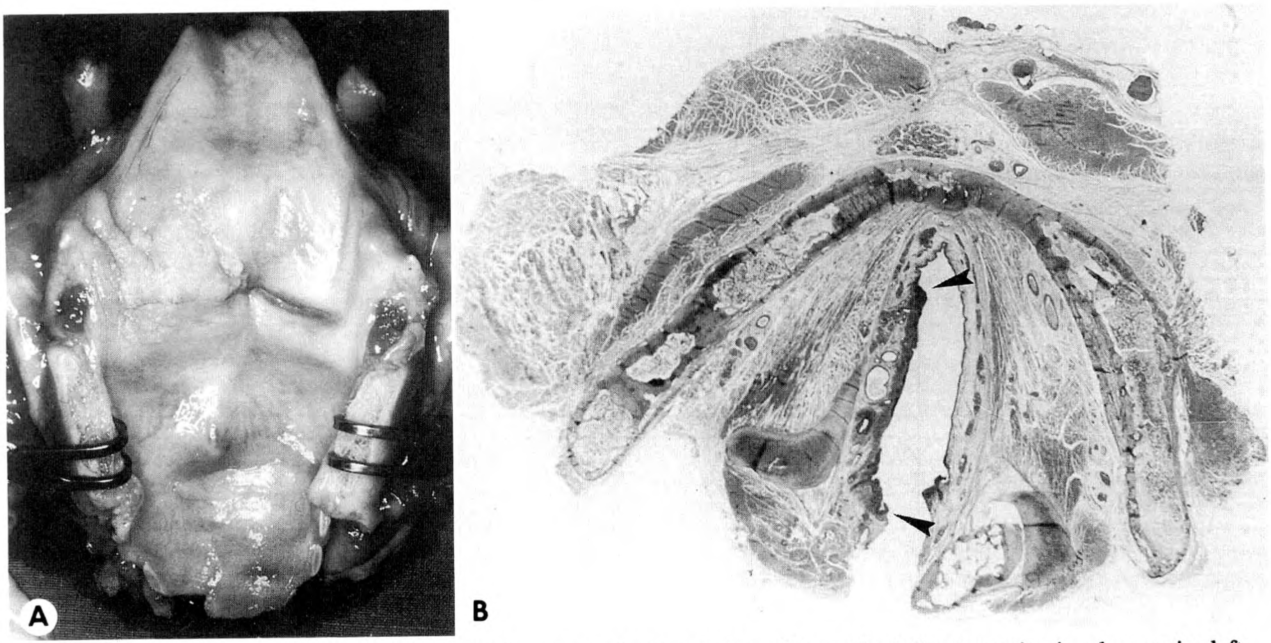

Fig 1. A) Laryngectomy specimen. Flat, whitish, granular-looking lesion with poorly defined margins involves entire left ventricular fold and reaches true vocal cord. B) Whole organ histologic section at supraglottic level of specimen shown in A. Tumor was confined to surface epithelium and lamina propria (arrowheads).

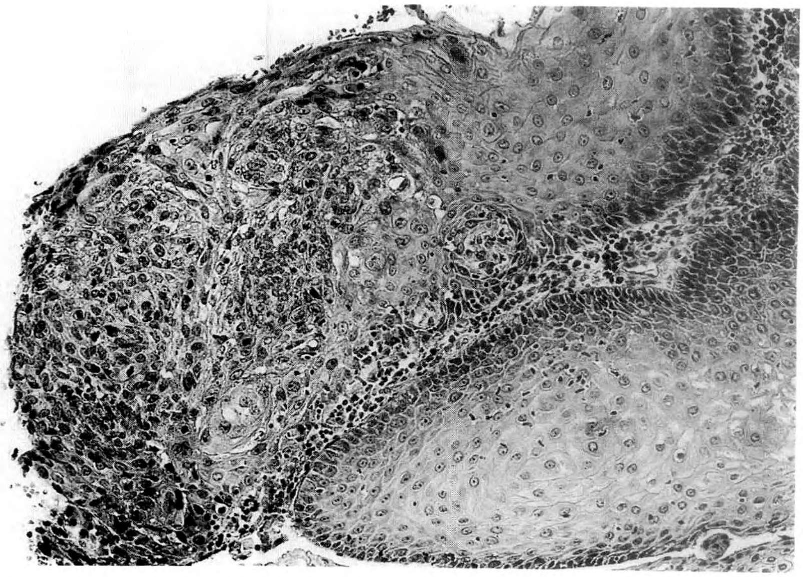

Fig 2. Carcinoma in situ.

early invasive squamous carcinoma. As a general rule, many pathologists will choose to err on the side of conservatism, and employ phrases such as "conclusive evidence of invasion is not identified" or "suspicious for superficial invasion" to convey their uncertainty with regard to whether or not a tumor has breached the basement membrane. As is probably intuitively obvious, the greater the abundance of tissue provided for histologic study, the greater the likelihood that a definitive diagnosis will be possible.

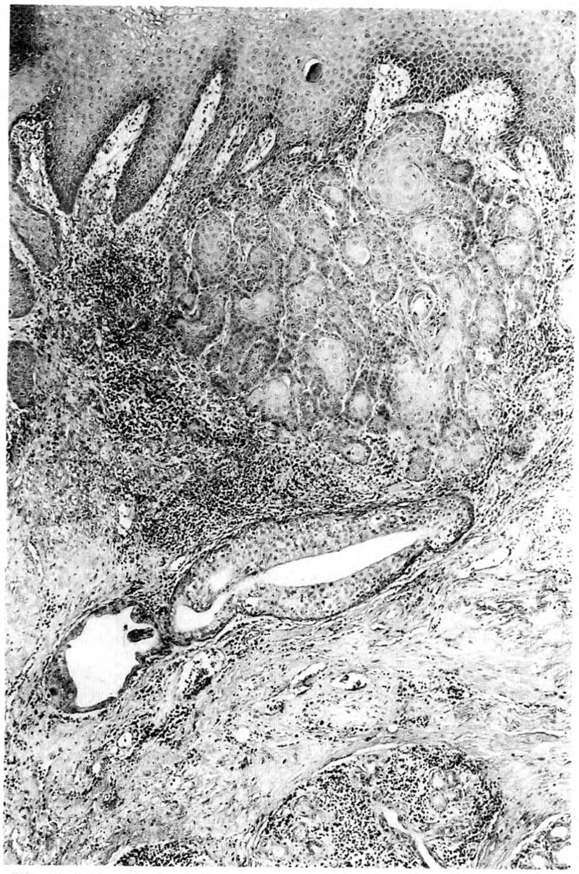

Fig 3. Microinvasive carcinoma. In early phase of invasion, irregular nests of well-differentiated squamous cells infiltrate lamina propria and evoke inflammatory response. Gland with squamous metaplasia is present near deeper carcinomatous cells.

To better clarify the pathologic definition of "early" cancer, we would like to emphasize some basic pathologic concepts, some of them based on our own experience. From a pathologic point of view, the natural history of human laryngeal cancer is based on a continuous morphologic spectrum of epithelial abnormalities and proliferative lesions. This spectrum encompasses intraepithelial lesions of the laryngeal mucosa potentially capable of progression into an invasive neoplasm, and "invasive" carcinoma.[6] The former lesions include "dysplasia," also termed as "laryngeal intraepithelial neoplasia," which commonly shows a variable degree (from mild to severe) of qualitative cellular or structural alterations, and "carcinoma in situ."[10]

Whether in dysplasia or laryngeal intraepithelial neoplasia or carcinoma in situ, the atypical change takes place in the epithelium, leaving the basement membrane intact. In contrast, invasive carcinoma violates the basement membrane and invades the underlying stroma, where it is potentially capable of penetrating lymphatic and blood vessels and subsequently forming lymph node or distant metastases. Thus, in contrast to the other previously mentioned alterations of the laryngeal epithelium, only invasive carcinomas have the capacity to metastasize via either lymphatic or blood vessels, according to the biologic concept of cancer.[6]

Pathologically, "invasive" carcinomas include those that infiltrate the lamina propria and underlying muscle or cartilaginous structures ("deeply infiltrating" carcinoma), as well as cases with only focal

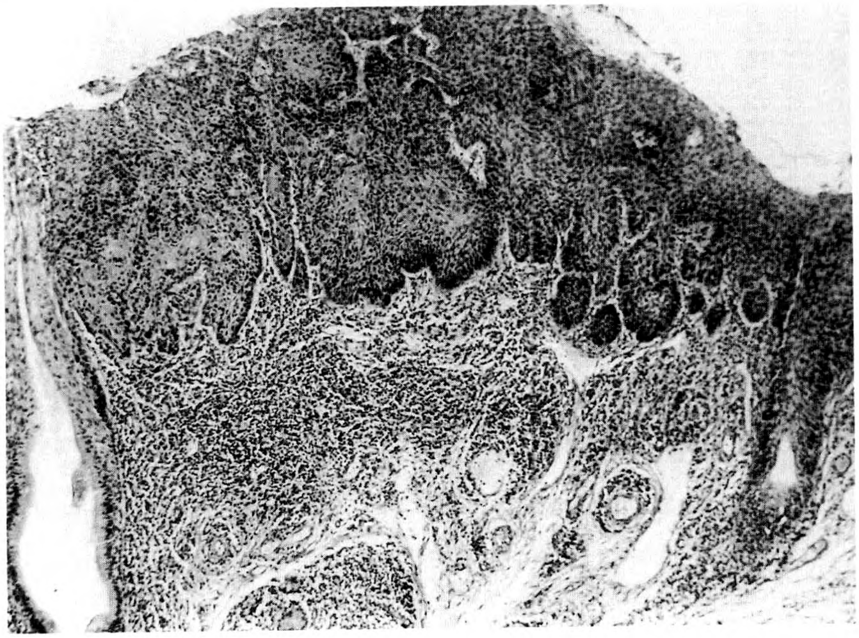

Fig 4. Superficial extending carcinoma (same case as Fig 1). This invasive, poorly differentiated carcinoma shows extensive involvement of lamina propria upper zones. Tumor, which is confined to lamina propria, is surrounded by exuberant lymphocyte-rich reaction.

early invasion of the lamina propria ("microinvasive" carcinoma).[8,11] In this latter variant the tumor is mostly an intraepithelial carcinoma (carcinoma in situ) associated with nests of malignant cells just below the basement membrane.[8,11]

In the last decade another variant of invasive carcinoma has been identified in the larynx, the so-called "superficial extending" carcinoma[12,13] (Fig 1). By definition, it represents an invasive carcinoma with prominent invasive growth through the basement membrane into the lamina propria, associated with extensive lateral spread limited to either the epithelium or lamina propria. The pathologic definition of "superficial extending" carcinoma is not influenced by the occurrence of lymph vessel invasion or lymph node metastasis.[12,13] Histologically, "superficial extending" carcinoma may be easily separated from a "microinvasive" carcinoma by the presence in superficial extending carcinoma of extensive invasion of the lamina propria, whereas in microinvasive carcinoma there is a prominent in situ component with only focal invasion of the lamina propria.[13] Intriguingly, multicentric lesions, "dysplasia or carcinoma in situ," "microinvasive" carcinoma, "superficial extending" carcinoma, and "deeply infiltrating" carcinoma can coexist in one and the same surgical specimen.[13]

Among the above-mentioned architectural and histopathologic lesions, "microinvasive" carcinoma[8,11] and "superficial extending" carcinoma[12,13] must be included under the term "early" laryngeal cancer,[14,15] ie, disease showing a preferential superficial type of

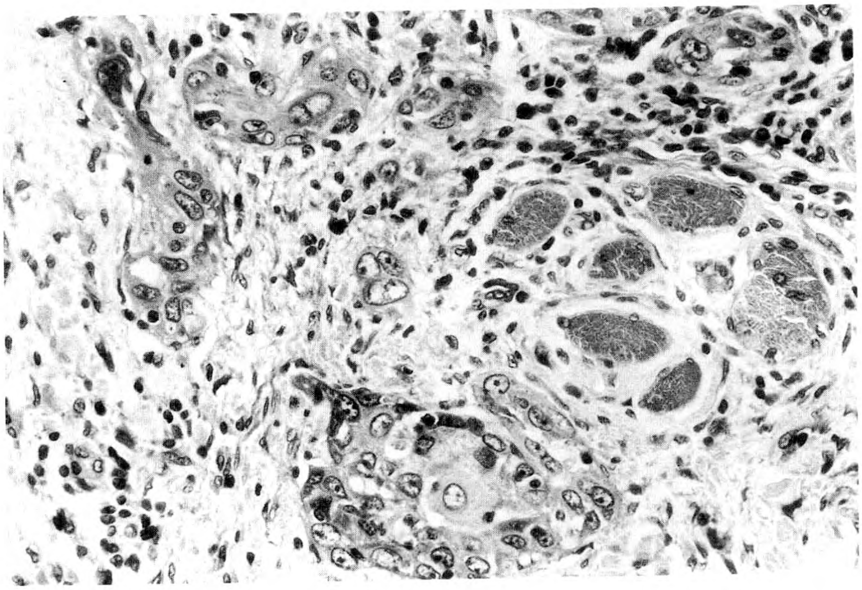

Fig 5. Deeply invasive carcinoma. Irregular sheets of malignant squamous cells infiltrate muscle deeply.

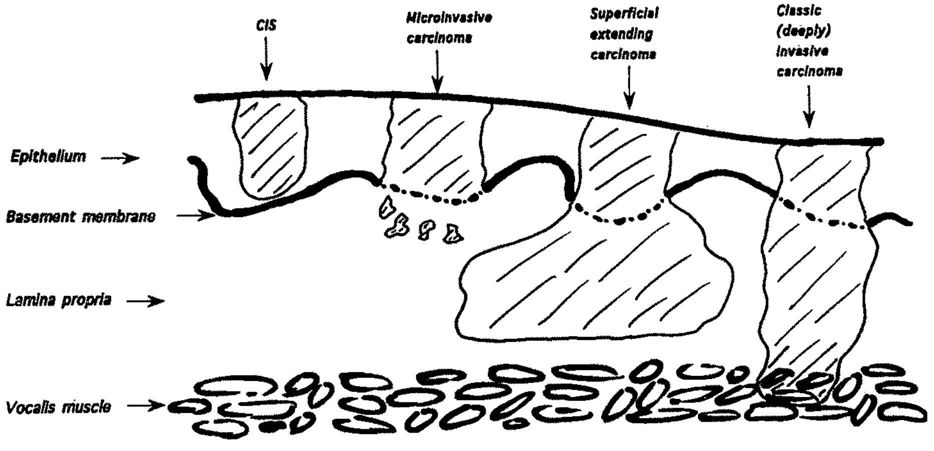

Fig 6. Schematic drawing of true vocal cord illustrating various types of carcinoma.

growth as opposed to tumors showing a more deeply infiltrative growth.[16] After the removal of the entire lesion and microscopic pathologic analysis,[6] the term "early" laryngeal cancer should be restricted to those "invasive" carcinomas that do not invade muscle or cartilaginous structures.[6] In other words, "early" laryngeal cancer is a superficial lesion that involves the lamina propria, thus representing more than a "carcinoma in situ" but less than a "deeply infiltrating" carcinoma.[6] Also, "superficial extending" carcinoma qualifies as an "early" cancer.

In conclusion, to avoid an inadequate or overzealous approach to treatment and prognosis, a clinical diagnosis of "early" invasive laryngeal cancer should be substantiated by a careful microscopic pathologic evaluation, which can exclude both carcinoma in situ and, particularly, deeply infiltrating carcinoma.

As for the *biologic definition*, early invasive cancer belongs to stage II, whereas carcinoma in situ comes under stage I.[17] It has to be stressed that "early" invasive carcinoma implies the concept of a lesion that has breached the basement membrane, while "carcinoma in situ" is by contrast an intraepithelial form of early cancer (in fact, the earliest form recognizable) in which the tumor cells are still confined by an intact basement membrane. Carcinoma in situ is, therefore, a preinvasive lesion that has not yet acquired the capacity for metastasis, as it is denied access to the lymphatic system by virtue of its confinement by the epithelial basement membrane. This is a critical point, producing important controversies in the definition of early glottic cancer between clinicians on the one hand and pathologists and biologists on the other.

Whereas "early" glottic cancer is synonymous with localized cancer, "early" cancer of the supraglottis may also invade lymphatic channels and metastasize to the neck. In fact, lymph node metastases occur in approximately 20% of T1 "early" supraglottic cancers.[18] It is therefore apparent that some seemingly "early" supraglottic cancers are not always localized but rather may be in an advanced stage, if they have metastasized.

In summary, "early" laryngeal cancers are "invasive" carcinomas ("microinvasive" or "superficial extending" carcinomas) confined to the lamina propria, but potentially capable of forming lymph node metastases (Figs 2-6).

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VESTIBULAR ADAPTATION

The symposium Vestibular Adaptation will be held May 23-25, 1996, in Santa Monica, California. For more information, contact Marjorie Harris, Conference Coordinator, 15-50 Rehabilitation Center, UCLA School of Medicine, Los Angeles, CA 90095-1651; telephone 310-825-5131; fax 310-206-2327.

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MIDDLE EAR RECONSTRUCTION BY TYMPANO-OSSICULAR ALLOGRAFTS

The Third International Antwerp Course on Middle Ear Reconstruction by Tympano-ossicular Allografts will be held (in English) on May 20-21, 1996, in Antwerp, Belgium. For further information, contact Prof Dr P. Van de Heyning, ENT Secretariat Sonja Beckers, University Hospital Antwerp, Wilrijkstraat 10, 2650 Edegem, Belgium; telephone **/32/3/821.34.51; fax **/32/3/825.05.36.

LETTERS TO THE EDITOR

To the Editor:

In August 1995, Pollack et al cast doubt upon the idea that papillary temporal bone tumors arise from the endolymphatic system (Are Papillary Adenomas Endolymphatic Sac Tumors? *Ann Otol Rhinol Laryngol* 1995;104:613-9). While two of their points are certainly logical, have some substance, and carry some weight, two other important main points seem to me to have very major weaknesses.

The authors indicate that the tumor radiologically appeared to arise in the infralabyrinthine area, inferior enough that it did not seem to arise from the endolymphatic sac, and this finding is an important basis for their argument. However, the patient developed Meniere's-type symptoms a year and a half before the tumor was first examined by computed tomography (CT), and these symptoms progressed to total deafness over 4 years. The time course of the auditory-vestibular symptoms and signs correlate with the progression of the tumor. Thus, these symptoms seem to have been caused by involvement of some portion of the labyrinth by the tumor, despite any suggestions to the contrary by the CT results. If the tumor *involved* the labyrinth very early in its growth, why, then, couldn't it have *arisen* from the labyrinth?

If one claims that the tumor did not involve the labyrinth early in its growth, one then needs to postulate some other unrelated disease, condition, or process to explain the symptoms. To postulate two unrelated diseases affecting the inner ear area, with these two diseases coincidentally developing and evolving *quite simultaneously*, is highly unlikely and is at odds with the principle of diagnostic parsimony (or Occam's razor as applied to medicine, à la Dr William Osler). This point becomes more compelling when one considers together the features of the dozens of other cases of papillary temporal bone tumors that have been reported. Virtually all such patients have had auditory-vestibular symptoms and signs (surely resulting from their tumors) similar to those of the present patient. Therefore, to suggest that the present patient's symptoms were caused by something other than the tumor would probably cause Dr Osler to turn over in the grave.

The authors claim their immunostain results exclude origin of the tumor from the endolymphatic sac. A few immunostains, such as thyroglobulin and prostate-specific antigen, are highly specific for a particular site of origin for a carcinoma. Most are not, and this is particularly true for the stains in the authors' Table (keratin, vimentin, S-100 protein, glial fibrillary acidic protein). Moreover, the absence of a particular immunoreactivity in an individual tumor example is seldom compelling evidence of anything, particularly since there can be variable results from time to time in a given laboratory and, especially, variable results among different laboratories (examples of which we see frequently at the Institute). The variable results indicated in the authors' Table support this point. The immunostain results hardly even suggest anything, let alone prove anything, and this is an extremely weak argument. The authors state that "immunohistochemical investigation is the necessary prerequisite to assessing the real origin of a tumor" (p 618) and state that their results indicate that the described tumor did not originate from the endolymphatic sac. To give the immunostain results this status of primary importance in their argument, when actually these results are extremely weak evidence at best, tends to weaken their entire argument.

The finding of ciliated cells in the tumor is logical evidence against an endolymphatic origin. However, possibly these cells were from air cell epithelium that was entrapped in the tumor as it disrupted air cells (as it surely did). Ciliated cells must have been very scarce, because they were not observed in the light microscopy sections.

The apparent absence of tumor in the lumen of the endolymphatic sac at surgery indeed seems to be evidence against origin from the sac. Conceivably, the tumor could have arisen from the lower edge of the sac and grown virtually entirely outward into the petrous bone rather than growing into the lumen of the sac. However, I admit that this seems to be a weak counterargument. Nevertheless, although this latter argument of the authors somewhat supports their thesis, the bulk of the evidence from the dozens of reported cases of similar tumors still is in favor, I believe, of an endolymphatic origin for this type of tumor. The authors imply that a single case report by Hassard et al[1] is the sole basis for thinking that papillary temporal bone tumors arise from the endolymphatic sac. There is much more evidence than this, including other cases with surgical or direct histologic[2] evidence, supporting a sac origin for such tumors.

The authors state that "the theory that the endolymphatic sac is the origin of *all* [emphasis mine] papillary-cystic tumors (adenocarcinomas) should be questioned" (p 613). The implication is that perhaps these tumors have more than one histogenetic source. While this is conceivable, it seems quite unlikely. The tumors seem to constitute a well-defined clinicopathologic entity, and to suggest that such a single unusual entity has two separate origins (which are embryologically different) would certainly, once again, disturb Dr Osler. Moreover, if temporal bone air sac epithelium were a source for some of the tumors, why would the tumors seem to arise only from cells above the jugular bulb (or near that area)? Granted that many of these tumors have been so large when discovered that their exact site of origin cannot be pinpointed, there is no case that I am aware of that strongly suggests an origin significantly away from the endolymphatic sac area, and yet there are air cells well away from that area.

I am most especially interested in what would be the authors' hypothesis of the genesis of the auditory-vestibular symptoms in their patient, which correlated so well with the development of the tumor, if the early symptoms were not due to early involvement of the labyrinth by the tumor.

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To the Editor:

Thank you for the opportunity to reply to Dr Heffner's comment on our paper on papillary adenomas of the temporal bone. Dr Heffner argues that we would have to postulate a second, unrelated disease to explain the development of auditory-vestibular symptoms progressing parallel to the tumor growth when we claim that the tumor did not involve the labyrinth early in its growth. We do not think that we have to violate the principle of diagnostic parsimony (or cause Dr Osler to turn over in his grave) when we postulate that the tumor in our case arose from the pneumatic spaces on top of the jugular bulb.

The cochlea and the vestibular apparatus are not autonomous islands in the temporal bone. Their functional integrity, rather, depends on metabolic exchanges by various pathways, among which the most important ones are the internal auditory artery for arterial blood supply, the inferior cochlear vein for venous blood drainage, the cochlear aqueduct for communications between perilymph and cerebrospinal fluid, and the endolymphatic duct and sac (for drainage of endolymph?). Interestingly, all authors who consider papillary temporal bone adenomas as endolymphatic sac tumors take for granted that the occlusion of the endolymphatic sac induces endolymphatic hydrops and Meniere's-like auditory-vestibular symptoms. Experimental obliteration of the endolymphatic sac consistently induces hydrops in guinea pigs, but rarely in chinchillas and never in squirrel monkeys.[1] In human surgery, approaches to the internal auditory canal are described that intentionally sacrifice the endolymphatic duct and at the same time claim to preserve hearing.[2]

We consider the inferior cochlear vein as an at least equally valuable candidate for remotely induced auditory-vestibular symptoms. This vessel provides the major venous drainage of the inner ear; it runs parallel to the cochlear aqueduct and empties into the bulb of the jugular vein[3] at approximately the site that we suspect to be the point of origin of the tumor in our case. The few experimental data on the effects of obstruction of the inferior cochlear vein on inner ear function have revealed degeneration of the spiral ligament, stria, and outer hair cells,[4] as well as decreases of the endocochlear potential and changes in inner ear fluid pressure.[5] Admittedly, these results were also obtained in guinea pigs, but this hypothesis is not weaker than the postulated endolymphatic sac occlusion.

We agree with Dr Heffner's criticism that immunostains can reveal variable results in a given laboratory and in different laboratories. However, the immunohistochemical profile of our tumor indicates endodermal or ectodermal, and not neuroectodermal, origin of the tissue. Dr Heffner's hypothesis on the probable endolymphatic sac origin of low-grade adenocarcinoma[6] is also based on, in his words, "the extremely weak evidence" of light microscopy, electron microscopy, and immunohistochemistry.

In regard to the ciliated cells found in the tumor of our patient, the tumor showed characteristics of benignity, and therefore, we diagnosed an adenoma. The very high degree of differentiation of the tumor cells does not exclude their nature of normal air cell epithelium. For that reason, it is not possible to distinguish between normal and tumor cells by morphology alone without immunohistochemistry. It is improbable that the scarce but clearly visible ciliated cells found in our specimen originated from air cell epithelium entrapped in the tumor, because all of our specimens were selected on semithin sections from tumor papillae.

One of the main pieces of evidence in favor of the origin of the papillary adenoma on top of the jugular bulb is given by surgery. The subtotal petrosectomy used in our patient permitted exposure of the entire endolymphatic sac and duct, as well as the complete jugular bulb. The tumor removed in our patient was sitting on top of the jugular bulb and did not destroy the endolymphatic duct and did not invade or originate from the lumen of the sac.

In summary, we are grateful to Dr Heffner (and Dr Megerian, in the January 1996 issue of the *Annals*) for their comments on our paper. They show how difficult it is to determine the exact origin of papillary-cystic temporal bone tumors. The pathogenesis of the clinical symptoms remains rather speculative and does not allow us to definitely decide pro or contra an endolymphatic sac origin; immunostain results and electron microscopic findings suggest rather than prove anything. The hardest facts are the surgical findings, which may vary according to the approach used for tumor removal. For these reasons and in view of the multiple and various types of tumors that are observed in von Hippel–Lindau disease, the exact origin of papillary adenomas of the temporal bone is still open to discussion.

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BRIEF SUMMARY

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CONTRAINDICATIONS: Clarithromycin is contraindicated in patients with a known hypersensitivity to clarithromycin, erythromycin, or any of the macrolide antibiotics.

Clarithromycin is contraindicated in patients receiving terfenadine therapy who have preexisting cardiac abnormalities (arrhythmia, bradycardia, QT interval prolongation, ischemic heart disease, congestive heart failure, etc.) or electrolyte disturbances. (See **PRECAUTIONS**-*Drug Interactions*.)

WARNINGS: CLARITHROMYCIN SHOULD NOT BE USED IN PREGNANT WOMEN EXCEPT IN CLINICAL CIRCUMSTANCES WHERE NO ALTERNATIVE THERAPY IS APPROPRIATE. IF PREGNANCY OCCURS WHILE TAKING THIS DRUG, THE PATIENT SHOULD BE APPRISED OF THE POTENTIAL HAZARD TO THE FETUS. CLARITHROMYCIN HAS DEMONSTRATED ADVERSE EFFECTS OF PREGNANCY OUTCOME AND/OR EMBRYO-FETAL DEVELOPMENT IN MONKEYS, RATS, MICE, AND RABBITS AT DOSES THAT PRODUCED PLASMA LEVELS 2 TO 17 TIMES THE SERUM LEVELS ACHIEVED IN HUMANS TREATED AT THE MAXIMUM RECOMMENDED HUMAN DOSES. (See **PRECAUTIONS** - *Pregnancy*.)

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including clarithromycin, and may range in severity from mild to life threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of "antibiotic-associated colitis".

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to discontinuation of the drug alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an antibacterial drug clinically effective against *Clostridium difficile* colitis.

PRECAUTIONS - *General:* Clarithromycin is principally excreted via the liver and kidney. Clarithromycin may be administered without dosage adjustment to patients with hepatic impairment and normal renal function. However, in the presence of severe renal impairment with or without coexisting hepatic impairment, decreased dosage or prolonged dosing intervals may be appropriate.

Information to Patients: BIAXIN tablets and oral suspension can be taken with or without food and can be taken with milk. Do **NOT** refrigerate the suspension.

Drug Interactions: Clarithromycin use in patients who are receiving theophylline may be associated with an increase of serum theophylline concentrations. Monitoring of serum theophylline concentrations should be considered for patients receiving high doses of theophylline or with baseline concentrations in the upper therapeutic range. In two studies in which theophylline was administered with clarithromycin (a theophylline sustained-release formulation was dosed at either 6.5 mg/kg or 12 mg/kg together with 250 or 500 mg q12h clarithromycin), the steady-state levels of C_{max}, C_{min}, and the area under the serum concentration time curve (AUC) of theophylline increased about 20%.

Concomitant administration of single doses of clarithromycin and carbamazepine has been shown to result in increased plasma concentrations of carbamazepine. Blood level monitoring of carbamazepine may be considered.

When clarithromycin and terfenadine were coadministered, plasma concentrations of the active acid metabolite of terfenadine were threefold higher, on average, than the values observed when terfenadine was administered alone. The pharmacokinetics of clarithromycin and the 14-hydroxy-clarithromycin were not significantly affected by coadministration of terfenadine once clarithromycin reached steady-state conditions. The increase in the QT interval seen in association with the elevated terfenadine acid metabolite level is unlikely to be of clinical significance in healthy individuals. Clarithromycin should not be given to patients receiving terfenadine therapy who have preexisting cardiac abnormalities (arrhythmia, bradycardia, QT interval prolongation, ischemic heart disease, congestive heart failure, etc.) or electrolyte disturbances. (See **CONTRAINDICATIONS**.)

Simultaneous oral administration of BIAXIN tablets and zidovudine to HIV-infected adult patients resulted in decreased steady-state zidovudine concentrations. When 500 mg of clarithromycin were administered twice daily, steady-state zidovudine AUC was reduced by a mean of 12% (n=4). Individual values ranged from a decrease of 34% to an increase of 14%. Based on limited data in 24 patients, when Biaxin tablets were administered two to four hours prior to oral zidovudine, the steady-state zidovudine C_{max} was increased by approximately 2-fold, whereas the AUC was unaffected.

Simultaneous administration of BIAXIN tablets and didanosine to 12 HIV-infected adult patients resulted in no statistically significant change in didanosine pharmacokinetics.

Concomitant administration of fluconazole 200 mg daily and clarithromycin 500 mg twice daily to 21 healthy volunteers led to increases in the mean steady-state clarithromycin C_{min} and AUC of 33% and 18%, respectively. Steady-state concentrations of 14-OH clarithromycin were not significantly affected by concomitant administration of fluconazole.

Spontaneous reports in the post-marketing period suggest that concomitant administration of clarithromycin and oral anticoagulants may potentiate the effects of the oral anticoagulants. Prothrombin times should be carefully monitored while patients are receiving clarithromycin and oral anticoagulants simultaneously.

Elevated digoxin serum concentrations in patients receiving clarithromycin and digoxin concomitantly have also been reported in post-marketing surveillance. Some patients have shown clinical signs consistent with digoxin toxicity, including arrhythmias. Serum digoxin levels should be carefully monitored while patients are receiving digoxin and clarithromycin simultaneously.

The following drug interactions, other than increased serum concentrations of carbamazepine and active acid metabolite of terfenadine, have not been reported in clinical trials with clarithromycin; however, they have been observed with erythromycin products and/or with clarithromycin in post-marketing experience.

> Concurrent use of erythromycin or clarithromycin and ergotamine or dihydroergotamine has been associated in some patients with acute ergot toxicity characterized by severe peripheral vasospasm and dysesthesia.
>
> Erythromycin has been reported to decrease the clearance of triazolam and, thus, may increase the pharmacologic effect of triazolam. There have been post-marketing reports of drug interactions and CNS effects (e.g., somnolence and confusion) with the concomitant use of clarithromycin and triazolam.
>
> The use of erythromycin and clarithromycin in patients concurrently taking drugs metabolized by the cytochrome P450 system may be associated with elevations in serum levels of these other drugs. There have been reports of interactions of erythromycin and/or clarithromycin with carbamazepine, cyclosporine, hexobarbital, phenytoin, alfentanil, disopyramide, lovastatin, bromocriptine, valproate, terfenadine, cisapride, pimozide, and astemizole. Serum concentrations of drugs metabolized by the cytochrome P450 system should be monitored closely in patients concurrently receiving these drugs.

Carcinogenesis, Mutagenesis, Impairment of Fertility:
The following *in vitro* mutagenicity tests have been conducted with clarithromycin:

Salmonella/Mammalian Microsomes Test
Bacterial Induced Mutation Frequency Test
In Vitro Chromosome Aberration Test
Rat Hepatocyte DNA Synthesis Assay
Mouse Lymphoma Assay
Mouse Dominant Lethal Study
Mouse Micronucleus Test

All tests had negative results except the *In Vitro* Chromosome Aberration Test which was weakly positive in one test and negative in another.

In addition, a Bacterial Reverse-Mutation Test (Ames Test) has been performed on clarithromycin metabolites with negative results.

Fertility and reproduction studies have shown that daily doses of up to 160 mg/kg/day (1.3 times the recommended maximum human dose based on mg/m^2) to male and female rats caused no adverse effects on the estrous cycle, fertility, parturition, or number and viability of offspring. Plasma levels in rats after 150 mg/kg/day were 2 times the human serum levels.

In the 150 mg/kg/day monkey studies, plasma levels were 3 times the human serum levels. When given orally at 150 mg/kg/day (2.4 times the recommended maximum human dose based on mg/m^2), clarithromycin was shown to produce embryonic loss in monkeys. This effect has been attributed to marked maternal toxicity of the drug at this high dose.

In rabbits, *in utero* fetal loss occurred at an intravenous dose of 33 mg/m^2, which is 17 times less than the maximum proposed human oral daily dose of 618 mg/m^2.

Long-term studies in animals have not been performed to evaluate the carcinogenic potential of clarithromycin.

Pregnancy: Teratogenic Effects. Pregnancy Category C.
Four teratogenicity studies in rats (three with oral doses and one with intravenous doses up to 160 mg/kg/day administered during the period of major organogenesis) and two in rabbits at oral doses up to 125 mg/kg/day (approximately 2 times the recommended maximum human dose based on mg/m^2) or intravenous doses of 30 mg/kg/day administered during gestation days 6 to 18 failed to demonstrate any teratogenicity from clarithromycin. Two additional oral studies in a different rat strain at similar doses and similar conditions demonstrated a low incidence of cardiovascular anomalies at doses of 150 mg/kg/day administered during gestation days 6 to 15. Plasma levels after 150 mg/kg/day were 2 times the human serum levels. Four studies in mice revealed a variable incidence of cleft palate following oral doses of 1000 mg/kg/day (2 and 4 times the recommended maximum human dose based on mg/m^2, respectively) during gestation days 6 to 15. Cleft palate was also seen at 500 mg/kg/day. The 1000 mg/kg/day exposure resulted in plasma levels 17 times the human serum levels. In monkeys, an oral dose of 70 mg/kg/day (an approximate equidose of the recommended maximum human dose based on mg/m^2) produced fetal growth retardation at plasma levels that were 2 times the human serum levels.

There are no adequate and well-controlled studies in pregnant women. Clarithromycin should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. (See **WARNINGS**.)

Nursing Mothers: It is not known whether clarithromycin is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when clarithromycin is administered to a nursing woman. It is known that clarithromycin is excreted in the milk of lactating animals and that other drugs of this class are excreted in human milk. Preweaned rats, exposed indirectly via consumption of milk from dams treated with 150 mg/kg/day for 3 weeks, were not adversely affected, despite data indicating higher drug levels in milk than in plasma.

Pediatric Use: Safety and effectiveness of clarithromycin in children under 6 months of age have not been established. Neonatal and juvenile animals tolerated clarithromycin in a manner similar to adult animals. Young animals were slightly more intolerant to acute overdosage and to subtle reductions in erythrocytes, platelets and leukocytes but were less sensitive to toxicity in the liver, kidney, thymus, and genitalia.

Geriatric Use: In a steady-state study in which healthy elderly subjects (age 65 to 81 years old) were given 500 mg every 12 hours, the maximum serum concentrations and area under the curves of clarithromycin and 14-OH clarithromycin were increased compared to those achieved in healthy young adults. These changes in pharmacokinetics parallel known age-related decreases in renal function. In clinical trials, elderly patients did not have an increased incidence of adverse events when compared to younger patients. Dosage adjustment should be considered in elderly patients with severe renal impairment.

ADVERSE REACTIONS

The majority of side effects observed in clinical trials were of a mild and transient nature. Fewer than 3% of adult patients without mycobacterial infections and fewer than 2% of pediatric patients without mycobacterial infections discontinued therapy because of drug-related side effects.

The most frequently reported events in adults were diarrhea (3%), nausea (3%), abnormal taste (3%), dyspepsia (2%), abdominal pain/discomfort (2%), and headache (2%). In pediatric patients, the most frequently reported events were diarrhea (6%), vomiting (6%), abdominal pain (3%), rash (3%), and headache (2%). Most of these events were described as mild or moderate in severity. Of the reported adverse events, only 1% was described as severe.

In pneumonia studies conducted in adults comparing clarithromycin to erythromycin base or erythromycin stearate, there were fewer adverse events involving the digestive system in clarithromycin-treated patients compared to erythromycin-treated patients (13% vs 32%; $p<0.01$). Twenty percent of erythromycin-treated patients discontinued therapy due to adverse events compared to 4% of clarithromycin-treated patients.

In two U.S. studies of acute otitis media comparing clarithromycin to amoxicillin/potassium clavulanate in pediatric patients, there were fewer adverse events involving the digestive system in clarithromycin-treated patients compared to amoxicillin/potassium clavulanate-treated patients (21% vs. 40%, $p<0.001$). One-third as many clarithromycin-treated patients reported diarrhea as did amoxicillin/potassium clavulanate-treated patients.

Post-Marketing Experience:
Allergic reactions ranging from urticaria and mild skin eruptions to rare cases of anaphylaxis and Stevens-Johnson syndrome have occurred. Other spontaneously reported adverse events include glossitis, stomatitis, oral moniliasis, vomiting, and dizziness. There have been isolated reports of hearing loss, which is usually reversible, occurring chiefly in elderly women. Reports of alterations of the sense of smell, usually in conjunction with taste perversion have also been reported.

Transient CNS events including behavioral changes, confusional states, depersonalization, disorientation, hallucinations, insomnia, nightmares, tinnitus, and vertigo have been reported during post-marketing surveillance. Events usually resolve quickly with discontinuation of the drug.

Hepatic dysfunction, including increased liver enzymes, and hepatocellular and/or cholestatic hepatitis, with or without jaundice, has been infrequently reported with clarithromycin. This hepatic dysfunction may be severe and is usually reversible. In very rare instances, hepatic failure with fatal outcome has been reported and generally has been associated with serious underlying diseases and/or concomitant medications.

Rarely, erythromycin and clarithromycin have been associated with ventricular arrhythmias, including ventricular tachycardia and torsades de pointes, in individuals with prolonged QT_C intervals.

Changes in Laboratory Values: Changes in laboratory values with possible clinical significance were as follows:

Hepatic - elevated SGPT (ALT) < 1%; SGOT (AST) < 1%; GGT < 1%; alkaline phosphatase <1%; LDH < 1%; total bilirubin < 1%

Hematologic - decreased WBC < 1%; elevated prothrombin time 1%

Renal - elevated BUN 4%; elevated serum creatinine < 1%

GGT, alkaline phosphatase, and prothrombin time data are from adult studies only.

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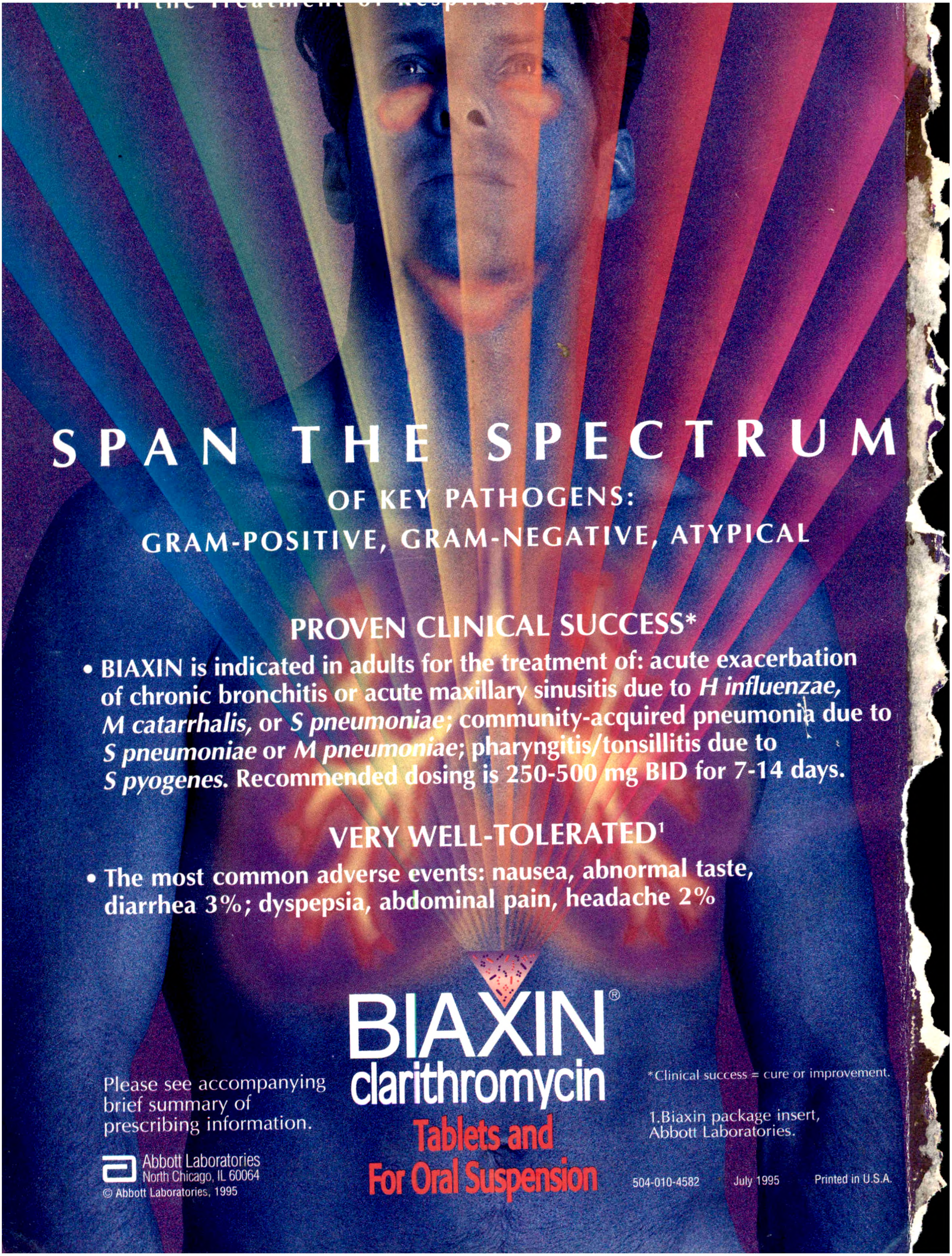

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SPAN THE SPECTRUM

OF KEY PATHOGENS:
GRAM-POSITIVE, GRAM-NEGATIVE, ATYPICAL

PROVEN CLINICAL SUCCESS*

- BIAXIN is indicated in adults for the treatment of: acute exacerbation of chronic bronchitis or acute maxillary sinusitis due to *H influenzae, M catarrhalis,* or *S pneumoniae*; community-acquired pneumonia due to *S pneumoniae* or *M pneumoniae*; pharyngitis/tonsillitis due to *S pyogenes.* Recommended dosing is 250-500 mg BID for 7-14 days.

VERY WELL-TOLERATED[1]

- The most common adverse events: nausea, abnormal taste, diarrhea 3%; dyspepsia, abdominal pain, headache 2%

BIAXIN®
clarithromycin
Tablets and
For Oral Suspension

Please see accompanying brief summary of prescribing information.

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*Clinical success = cure or improvement.

[1] Biaxin package insert, Abbott Laboratories.

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